COMPARATIVE STUDIES UPON CANCER CELLS AND NORMAL CELLS.

II. The Character of Growth in Vitro with Special Reference to Cell Division.*

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Plates 98 to 100.

In presenting this report it is realized that general conclusions upon the nature of cancer based upon the observation of cells cultivated under artificial conditions should be formulated with hesitation. It may be pointed out, however, that our previous studies, as well as a part of the present investigation, were undertaken to show the similarity in the behavior of tumor cells *in vitro* and *in vivo*.

A series of comparative studies upon cancer cells and normal cells have been made in this laboratory during the past year. The first report upon this work dealt with the susceptibility to heat of sarcoma cells and connective tissue cells (1). The present report will be limited to a consideration of the general character of growth *in vitro* of the same two tissues with special reference to cell division.

GENERAL CHARACTER OF GROWTH.

Recent papers on the subject render unnecessary a detailed description of the general phenomena of growth in tissue cultures and the technique of the method. We have used two transplantable tumors as material for growing malignant tumor cells; one a spindle cell sarcoma of the rat, the other a mixed cell sarcoma of the mouse. Normal connective tissue cells were grown chiefly from small pieces of blood vessel removed from growing or adult animals.

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Primary cultures of cells from the two kinds of sarcoma and the blood vessel differ rather strikingly, though not fundamentally, as a brief description of each will show. A piece of blood vessel placed in a hanging drop of plasma shows at the end of twenty-four hours few if any cell outgrowths and forty-eight hours may elapse before activity is noted; but at the end of the third or fourth day a fairly dense halo of cells is seen surrounding the tissue fragment. Growth, with slow radial extension of the cells into the coagulum, may continue for several days longer. The cells are for the most part spindle in form, with long, delicate processes at either extremity. There may be, however, considerable variation in morphology, the size and shape of the cells depending largely on their position in the hanging drop: those creeping along the cover-glass or upon the surface of the coagulum may be flattened and polygonal in outline, while those wandering through the clot are more compact, spindle-shaped, triangular, or radiate in form, and possess long processes.[1] Karyokinetic figures are occasionally seen. The accumulation of fat in the cells proceeds slowly reaching a maximum after five or six days.

Although the sequence of changes in sarcoma cultures is much the same as in cultures of blood vessel, the rate of their occurrence is more rapid. There is practically no latent period; and cells begin to migrate within a few hours. At the end of twenty-four hours the culture has often reached the height of its activity and numerous mitotic figures are seen (figure 1). By the end of the second day many of the actively migrating cells have reached the edge of the drop of plasma. The cells have become loaded with fat, and growth may cease unless the tissue is transferred to fresh culture media.

It is thus seen that in primary cultures the differences in the general character of growth of sarcoma and connective tissue are such as might have been anticipated, being, apart from the morphol-

[1] Failure to recognize the influence of mechanical factors upon the morphology of cells growing in cultures may easily lead to erroneous interpretations; several types of tissue cells may, for example, be described in a preparation in which only one kind of cell is growing. This source of error is illustrated by figure 13 in a recent paper by Lambert and Hanes (Lambert, R. A., and Hanes, F. M., *Virchows Arch. f. path. Anat., loc. cit.*, p. 106.)

ogy of the cells, chiefly in the rate and extent of growth and ameboid wandering.

In secondary and subsequent cultures differences in rate of activity are likewise noticeable, but the relations are considerably altered. Sarcoma cells grow less vigorously, cell wandering and multiplication being less marked than before. In the case of mouse sarcoma we have found it difficult to propagate the cells through subcultures for any length of time. Connective tissue cells, on the contrary, grow more vigorously than in primary cultures. The cells begin to wander out almost immediately. Mitotic figures are often extremely numerous (figure 2). In one preparation more than a hundred cells were observed undergoing mitotic division simultaneously. Apparently the resting connective tissue cells of the vessel wall have become transformed within a few days into actively proliferating fibroblasts, similar to what occurs during the reparative process following a wound in the body. It is conceivable that the mechanism of the reaction is the same in both cases.

That secondary cultures of connective tissue cells are more regularly successful, exhibit many more mitoses, and often show a more extensive growth than secondary cultures of sarcoma, has been shown conclusively in a large number of experiments. Several explanations of this fact, each of which upon analysis seems inadequate, have suggested themselves: (1) that tumor cells are in general less hardy than normal cells and the failure to thrive under artificial conditions is but an evidence of a hypothetical lowered vitality; (2) that tumor tissue requires a larger food supply than normal connective tissue and the food supply in tissue cultures is insufficient for the continuous active growth of sarcoma cells; (3) that some substance, as, for example, the internal secretion of one or more glands, upon which the continuous multiplication of tumor cells may depend, is present in insufficient amount or is too irregularly supplied in cultures.

The first explanation, which postulates a low resistance to injuries of all kinds on the part of tumor cells, has some support. Ribbert (2) some years ago expressed this view, basing his opinion on the clinical observation of the effects of radium, x-ray, etc., which exhibit a more destructive action upon cancer cells than upon

the adjacent normal tissues, and he suggested that the low vitality of the cells was caused by a poor circulation. In a recent paper (1) we described experiments showing that rat sarcoma cells are less resistant to heat than are normal connective tissue cells. On the other hand, the vitality exhibited by the cells of rat and mouse tumors under different conditions of transplantation indicates at least a rather high degree of resistance to mechanical injuries. Furthermore, the greater resistance to low temperatures of mouse tumor cells, as compared with normal body cells, can hardly be questioned.

The second explanation involves the assumption that tumor tissue requires more food than an equal quantity of normal tissue. Experimental data on this point are meager; such as we have, however, do not support the notion. Cramer and Pringle (3), for example, have shown that in the case of a rat sarcoma less nitrogen is required to build up a certain amount of tumor than to build up an equal weight of somatic tissue.

Our knowledge at present of the factors controlling the growth of normal tissues is so limited that the general idea contained in the third explanation, namely, that the unlimited growth of tumor cells is dependent upon some body secretion which is insufficiently supplied in cultures, can hardly be discussed. While there is a general tendency to attribute to tumor cells an independence of body function, such an independence has not been proven.

It was mentioned in a former paper that we had been able, by successive transfers, to propagate rat connective tissue cells *in vitro* for more than three months, at which time the cells were still multiplying actively. No changes indicating degeneration from old age were observed. While this experiment covers too short a period to be of much significance, it suggests a reference to the point of the immortality, so called, of the cancer cell which the cancer transplantation experiments of the past twelve years tend to support. The fact that tumors arising in rats and mice have been propagated by successive transplantation to other hosts over a period several times that representing the life of the animal in which the tumor originated, has been emphasized. It will be interesting to see if we shall be able to cultivate normal cells (of the mouse, for

example) for a period exceeding the life of an individual of that species. It may thus be possible to determine whether or not the attribute of immortality is to be regarded as characteristic of the cancer cell.

CELL DIVISION.

The presence of atypical division figures in the cells of malignant new growths has been noted by a number of observers (Cornil, Klebs, von Hansemann (4), Galeotti (5), Farmer, Moore, and Walker (6), Bashford and Murray (7), and others.[2] The contributions of most interest, on account of the theoretical importance attached to the observations recorded, are those of von Hansemann, and Farmer, Moore, and Walker.

von Hansemann regarded the presence of atypical mitoses in tumors as an expression of a change in biological character that occurs when normal cells become anaplastic, or undifferentiated. It is the anaplastic cell, he maintains, which, upon receiving the proper stimulus, becomes the cancer cell.

Farmer, Moore, and Walker, who saw in the abnormal mitoses in tumors a striking resemblance to the type of division in reproductive tissue (heterotypical mitosis), attached another significance to the phenomenon. Their theory is as follows: The remote cause of cancer, which may be some form of chronic irritation, induces in normal cells a change in their mode of division which results eventually in a definite heterotypical karyokinesis. Cells exhibiting this type of division possess the inherent power of unlimited growth. The mechanism, then, of the development of cancer cells, consists essentially in the production of a change, through some unknown agency in the character of division in normal body cells. This theory invites attack first on account of the assumption that reproductive cells possess a power of unlimited infiltrative growth. In support of this assumption they refer to the parasitic growth assumed by the cells of the embryo sac in certain flowering plants. von Hansemann has criticized this example on the ground that such a parasitic growth bears no analogy to the invasive growth of malignant tumor cells in the body. It is sufficient to point out, as Bashford says, that there is no evidence either in histological studies or in experimental work, such as transplantation of testis and ovary or ligation of the vas deferens, that the reproductive cells of vertebrates possess the properties postulated in this theory.

The question as to whether the mitoses in cancer are definitely heterotypical, as Farmer, Moore, and Walker maintain, will be taken up later.

The work upon which we shall report at this time is concerned with a study of mitosis in stained and fresh cells in cultures of

[2] An exhaustive review of the subject with a complete bibliography is given by Wolff, J., Die Lehre von der Krebskrankheit von den ältesten Zeiten bis zur Gegenwart, Jena, 1907, i.

sarcoma and connective tissue. The method used permits of a more complete study of the process of cell division in vertebrate tissues than has hitherto been possible, since observations can be made upon living growing cells (figure 6). Through such observations serial pictures made from the same cell can be obtained, and the time required for division, which, as we shall show, differs markedly in normal and cancer cells, can be determined. The method also permits one to fix and stain a mitotic figure in any phase, thus making it possible to verify observations upon the living structure (figure 7). For this reason alone the method will appeal to those interested in a finer study of normal and pathological mitoses in the tissues of higher animals. It should be explained that with this method sections of the tissue and media are not necessary, and the cells are usually well separated in the plasma and are often so flattened against the cover-glass or on the surface of the coagulum that a determination of the number of chromosomes is no more difficult than in paraffin sections (figure 3, *c*).

The questions investigated have been (1) the character of the mitoses, and (2) the rate of division in sarcoma and connective tissue cells.

CHARACTER OF THE MITOSES.

According to von Hansemann (8) and others, atypical division figures in cancer cells may be of three kinds: (1) asymmetrical mitoses; (2) multipolar mitoses; and (3) hyper- and hypochromatic cells. Asymmetrical mitoses were regarded by von Hansemann as of most significance; they were found only in carcinoma. Distinct asymmetrical figures have not been found in our sarcoma preparations. Multipolar mitoses, however, were not infrequently encountered. A tripolar mitosis is shown in figure 5, *b*. That multipolar division of the nucleus may result in the formation of multinucleated cells, appears probable from observations to which reference will be made.

Abnormal pictures suggesting tetrapolar mitoses were occasionally noted in fresh cells but upon fixing and staining the cells it was seen that a lagging of chromosomes was responsible for the appearance. The cell in figure 4, *b*, and probably also the one in

figure 5, *a*, are instances of this. The cell in figure 4, *b*, had been under microscopical observation in the warm box for about thirty minutes; the chromosomes were distinctly visible in the equatorial plate stage. The mass of chromosomes began to break up but instead of a division into two equal groups with segregation at the poles of the cell, there seemed to be a general scattering. After a few minutes two irregular clumps could be made out situated near the poles; the remaining chromatin material could be seen with difficulty. The preparation was then fixed with formalin vapor and stained with iron hematoxylin.

Hyper- and hypochromatic mitoses were somewhat more common than multipolar figures (figures 1 and 4, *a*). Hyperchromatic cells seemed to be more numerous than cells with a diminished number of chromosomes. A large number of counts, however, was not made, since a study of this kind did not appear necessary in view of the studies of von Hansemann and his pupils, and the recent work of Bashford and Murray (9).

Their results indicate, in brief, that while a reduction in the number of chromosomes is often observed in cancer cells, the reduction is not an exact halving; furthermore, the chromosomes are not of the same character as those found in gametogenic cells. An analysis of Farmer, Moore, and Walker's papers shows that according to their own observations only a small percentage of the mitoses in cancer are definitely heterotypical. This fact in itself should at least lessen the force of their conclusions.

von Hansemann maintained that hypochromatic cells were developed in two ways: first, by asymmetrical division, and secondly, by a casting out and degeneration of chromosomes during division. The latter phenomenon we have observed in unstained cells. In the instances noted, one or more chromosomes were seen to be left behind as the two masses of chromatin separated in anaphase. The lost chromosomes could be followed long enough for the observer to be convinced that they did not enter into the formation of the daughter nuclei; but their ultimate fate was not determined with certainty. They appeared to become dissolved in the cell cytoplasm.

Amitotic division has been described as a fairly common occurrence in tumor cells and as taking place not infrequently in connective tissue cells. It seemed, therefore, of interest to search for evidences of the process in living cells. The ease with which mitosis may be studied in unstained preparations made it appear probable that stages in amitotic division, if present, might also be followed. However, after observations upon several hundred cells

undergoing division, an example of amitosis has not been made out in living cells. It is possible, of course, that the phenomenon might escape notice, especially in connective tissue cells where the nuclei are sometimes not distinct and the cell outlines not well defined. But even in stained preparations convincing figures indicating the formation of two cells following amitotic nuclear division were not found. Dumb-bell nuclei, as well as nuclei presenting knob-like prominences partially or completely constricted off, were seen both in tumor and connective tissue preparations (figure 8). Such a breaking up of the nucleus, however, seemed to result not in the formation of new cells but in the development of multinucleated cells of moderately large size.

We have made one observation which shows that cells with two nuclei, such as are seen not uncommonly in culture preparations, may be the result of mitotic division of the nucleus without cytoplasmic division. In the instance referred to, a cell under observation in a warm chamber exhibited all the phases of mitosis up to constriction and division of the cytoplasm. Within half an hour long pseudopods had been thrown out. At this point the preparation was fixed and stained and the fact established that two nuclei of normal size and appearance were present.

The question as to why cancer cells divide in an abnormal manner has interested those who have studied the phenomenon. The theories of von Hansemann and of Farmer, Moore, and Walker are largely concerned with the answer to this problem. The results of some experiments by Häcker (10) and Galeotti (5) are perhaps significant in this connection. Galeotti describes atypical mitoses similar to those seen in cancer cells in the epidermis of the salamander produced through the action of quinine, antipyrine, and other agents. Häcker obtained similar results by subjecting the eggs of cyclops to ether. The suggestion was made that in mouse cancer at least the atypical mitoses described might have been the result of ether narcosis. This idea was readily refuted by Bashford.

Ribbert (2), looking for a more tangible explanation, suggested that malnutrition occasioned by the poor circulation in tumors might be responsible for the abnormalities in division. It is doubtful if the present studies throw any light on the question. With Ribbert's explanation in mind it may be suggested that the conditions of food supply are the same for cultures of both kinds of tissue, in one of which abnormal mitoses are present, in the other, absent. It must be remembered, however, that abnormalities in structure, developed during the growth of cells in the body, could not be expected to disappear in a succeeding generation *in vitro*, even though the causative factor might conceivably be removed.

RATE OF DIVISION.

As indicated above, the chromatin material is clearly visible in the cell during division. The earliest changes in the nucleus are not always easily made out, but with proper lighting and practice in making observations the process can be followed through all phases.

We pointed out previously (11) that under constant conditions the time required for connective tissue cells of the rat to divide did not vary greatly. For example, at body temperature (38° C.) they require, as a rule, twenty-five to forty-five minutes. We found that the time required varied with the temperature, and that cells of different species show somewhat different time periods.

In the case of sarcoma cells, however, we have found that a marked variation in the division rate obtains, even among cells in the same preparation. The time is rarely shorter than that required by connective tissue cells, but two to four hours may be occupied in the process. Table I contains records of observations upon mitosis in ten sarcoma cells and an equal number of connective tissue cells.

TABLE I.

Rat Connective Tissue Cells.

No. of animal.	Temperature, C.	Days *in vitro*.	Mitosis began.	Separation of daughter chromosomes.	Beginning constriction of cytoplasm.	Division completed.	Time required in minutes.
1	34°	15	9.10	9.35	9.38	9.45	35
2	34°	15	9.20	9.51	9.54	10.01	40
3	34°	78	5.30	6.10	6.12	6.20	50
4	36°	15	8.35	9.07	9.09	9.15	40
5	36°	4	8.34	9.09	9.11	9.16	42
6	38°	20	3.45	4.07	4.10	4.13	28
7	38°	40	9.30	9.45	9.47	9.52	22
8	38°	20	11.03	11.21	11.24	11.29	26
9	39°	10	2.18	2.41	2.44	2.47	29
10	39°	10	2.10	2.26	2.28	2.31	21

Rat Sarcoma Cells.

1	35°	5	10.00	11.00	11.03	11.12	72
2	35°	3	9.15	10.34	10.36	10.40	85
3	37°	3	9.20	9.42	9.45	9.55	30
4	37°	4	8.03	Complete disintegration at 10:15			
5	37°	3	3.15	4.43	4.45	4.52	97
6	38°	3	7.20	7.38	7.40	7.45	25
7	38°	2	9.45	10.44	10.47	10.55	70
8	38°	2	10.20	10.45	10.49	11.04	44
9	38°	3	10.30	Complete disintegration at 12.30			
10	38°	8	2.10	2.25	2.27	2.34	24

Occasionally a cell is seen in which mitosis appears to progress normally to the formation of the equatorial plate and then stops; the nuclear material becomes gradually condensed into a translucent mass, and, after one to three hours rapid disintegration of the cell ensues. This phenomenon is striking. The cell suddenly bursts; a number of small rounded fragments constitute the debris. The course of division in the later phases in sarcoma cells is usually the same as in connective tissue cells; that is, within two or three minutes after the separation of the masses of daughter chromosomes, constriction of the cell body begins, division of the cytoplasm being completed in five to ten minutes. Pseudopods are immediately thrown out by the daughter cells, which within a half hour have become indistinguishable from the neighboring resting cells.

CONCLUSIONS.

1. In primary cultures sarcoma cells exhibit a much greater activity than do normal connective tissue cells grown from the adult blood vessel; there is a shorter latent period, ameboid phenomena are more marked, and cell multiplication proceeds more rapidly.

2. In secondary cultures sarcoma cells are less active than in primary cultures; connective tissue cells, on the other hand, show a markedly accelerated growth.

3. Connective tissue cells are more easily propagated over long periods *in vitro* than are sarcoma cells; they multiply actively in cultures more than three months old.

4. The method of tissue cultivation is well adapted to the study of normal and pathological cell division; the nuclear changes are easily discernible in the living cell as division proceeds, and staining methods may be applied to verify observations upon the unstained structures.

5. Atypical mitoses of several kinds are found in cultures of sarcoma cells but are not seen in growths of connective tissue.

6. The time required for division in rat connective tissue cells kept at body temperature (38° C.) varies within relatively narrow limits (twenty to fifty minutes); sarcoma cells, on the contrary, exhibit marked variations and several hours may be required.

7. In studies upon living cells amitotic division has not been observed in either normal or tumor tissue. Evidences of nuclear budding, however, with the formation of cells containing several nuclei of irregular size have been noted.[3] The development of a cell with two nuclei from a mononuclear cell by mitotic division of the nucleus without division of the cytoplasm has also been observed.

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EXPLANATION OF PLATES.

Plate 98.

Fig. 1. A field in a three day culture of mouse sarcoma. In the upper part of the picture cells are seen wandering along the cover-glass in an area from which fibrin has retracted. In the lower part cells are seen moving through the fibrin meshwork. Seven mitoses are visible; one, on the left, is a large hyperchromatic cell.

Fig. 2. An area containing a tongue-shaped group of cells in a fifteen day secondary culture of rat blood vessel. The four dividing cells represent several phases of karyokinesis: (*a*) prophase; (*b*) metaphase, with complete withdrawal of pseudopods; (*c*) telephase, showing segregation of daughter chromosomes, constriction of cytoplasm, and beginning extrusion of pseudopods; (*d*) two daughter cells immediately after division, showing compact, deeply staining nuclei.

[3] Cells of the type here referred to are not to be confused with the large multinucleated giant cells formed about foreign bodies or on the cover-glass in cultures of chick or rat spleen, the mechanism of the formation of which was discussed in a former paper (Lambert, R. A., *Jour. Exper. Med.*, 1912, xv, 510).

PLATE 99.

FIG. 3. Three mitoses from a four day preparation of rat sarcoma, showing no abnormality in structure; (*a*) a cell with the spindle peripherally situated; (*b*) normal mitosis; (*c*) a cell flattened on the cover-glass; the chromosomes are well separated.

FIG. 4. Three mitoses from a three day culture of rat sarcoma; (*a*) a large hyperchromatic cell; (*b*) mitosis with lagging chromosomes; (*c*) mitosis in a cell containing a large vacuole.

FIG. 5. Three cells from a five day growth of rat sarcoma; (*a*) bipolar mitosis with lagging chromosomes. This cell when fresh had the appearance of a tetrapolar mitosis; (*b*) tripolar mitosis; (*c*) a cell containing three newly formed nuclei, the result apparently of a tripolar mitosis without division of the cell cytoplasm.

PLATE 100.

FIG. 6. Four drawings at fifteen minute intervals of a rat connective tissue cell undergoing division, and a resting cell (*x*) for comparison. (*a*) Prophase; (*b*) metaphase; (*c*) telephase; (*d*) daughter cells fifteen minutes after division. In the living cell the chromosomes are visible in the cytoplasm on account of their slightly greater refractivity. They are represented here in dark color for the sake of clearness. The small white spaces in the cells are fat droplets.

FIG. 7. (*a*) Young connective tissue cells fixed and stained five minutes after division; (*b*) connective tissue cells fixed and stained thirty minutes after division; (*x*) resting cell for comparison.

FIG. 8. (*a*) Budding nuclei in sarcoma cells; (*b*) the same in connective tissue cells.

FIG. 1.

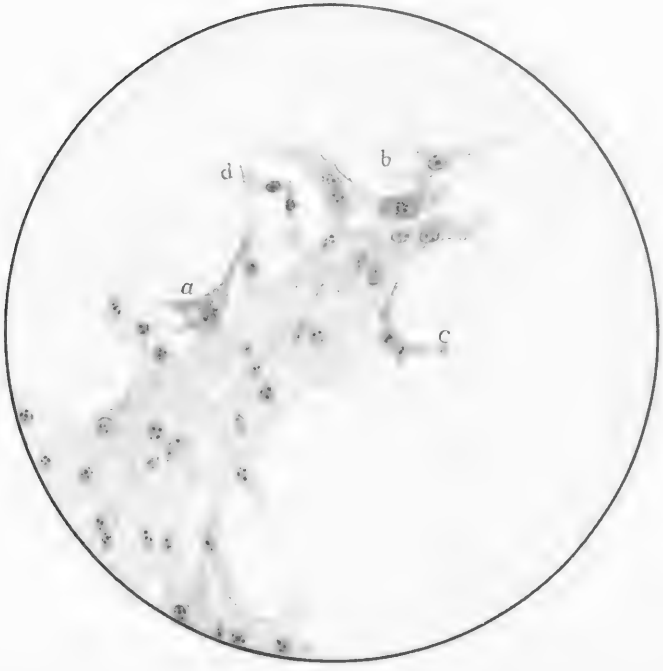
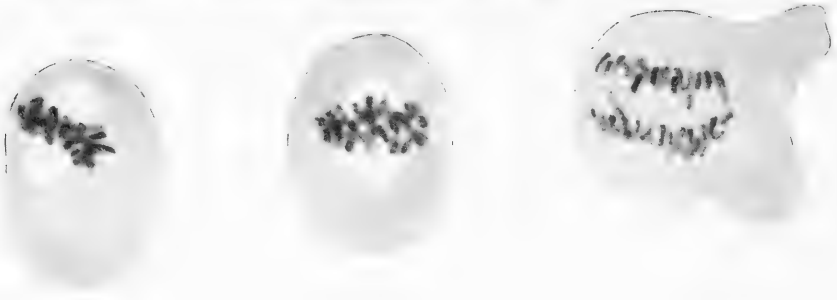


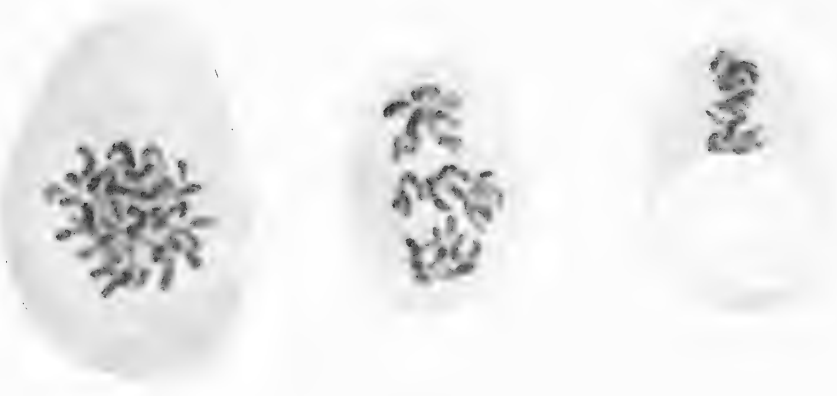
FIG. 2.

(Lambert : Cancer Cells and Normal Cells.)



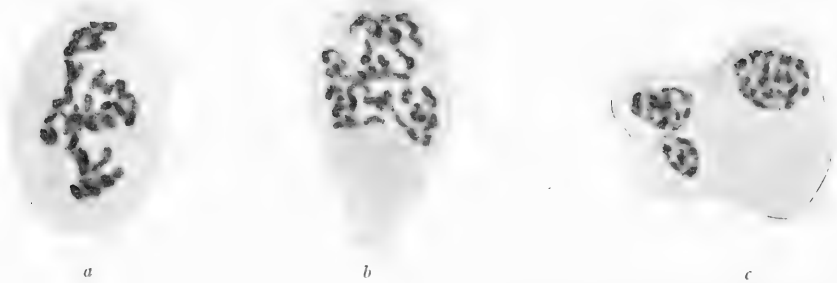
a *b* *c*

FIG. 3.



a *b* *c*

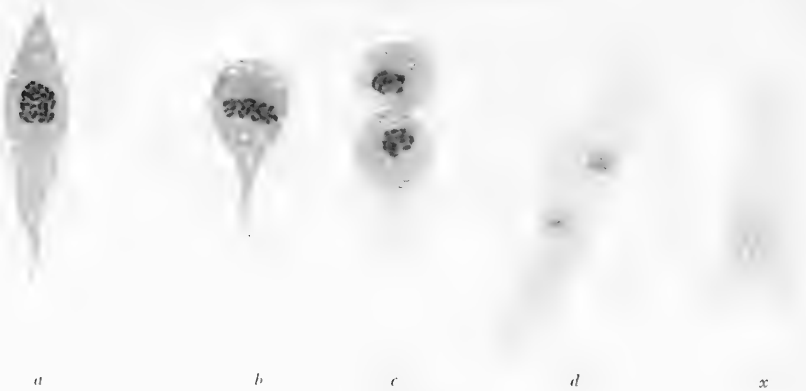
FIG. 4.



a *b* *c*

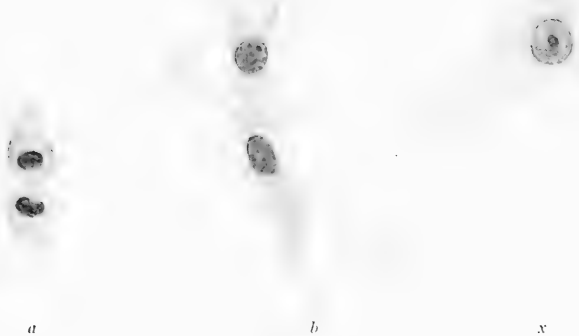
FIG. 5.

(Lambert: Cancer Cells and Normal Cells.)



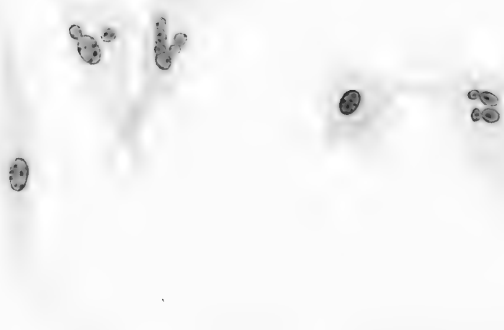
a *b* *c* *d* *x*

FIG. 6.



a *b* *x*

FIG. 7.



a *b*

FIG. 8.

(Lambert: Cancer Cells and Normal Cells)

THE BACTERIOLOGY AND VACCINE THERAPY OF DISTEMPER IN HORSES.*

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On September 5, 1910, an epidemic of distemper, also known as influenza or shipping fever, broke out among the horses of Squadron C of the National Guard of the State of New York. The symptoms, in brief, were a sudden onset with elevation of temperature of 105° to 106° F., marked weakness, constipation, partial or complete loss of appetite, and extreme depression. The pulse rose only slightly above normal, except in the last stages of the disease. The duration of the disease, on the average, was from seven to ten days. There were thirty-one cases in all, of which thirteen terminated fatally. The diagnosis of influenza or distemper was made.

On December 20, 1910, I was consulted with the view of studying the bacteriology of the disease. Thinking that the mouse might be an important factor in the spread of the epidemic, I autopsied a number of mice that were found dead in the stable.

Autopsy.—The peritoneums were hyperemic in spots and contained a small amount of viscid gelatinous fluid. All the organs were markedly congested, otherwise negative. Cultures from the blood and organs revealed a Gram-positive, spore-bearing, actively motile, non-encapsulated bacillus, which did not appear in chains, but singly and in pairs. Inoculation of this organism into mice, rats, guinea pigs, and rabbits proved non-fatal. I thus ruled out the anthrax bacillus as the causative agent. The microörganism isolated was a saprophyte.

That the epidemic was due to a contagion was evident, and that the contagion was of a bacterial nature was probable. Accordingly I withdrew from the jugular vein ten cubic centimeters of blood and inoculated it on various media. The results were negative.

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On January 2, 1911, another horse (No. 1) died. The autopsy revealed the morbid changes of influenza.

Autopsy of Horse 1. Body.—Well nourished; coat smooth and glossy. Fluid in thoracic and abdominal cavities normal in amount. *Lungs.*—Throughout both lungs are areas of varying size, showing the stage of red hepatization. *Trachea and larynx.*—Marked congestion of mucosa. *Heart.*—Somewhat enlarged and flabby. The myocardium is salmon colored in appearance and shows evidence of intense degeneration. No endocarditis present. A non-organized ante-mortem clot the size of a fist occupies the left auricle and ventricle. A much smaller ante-mortem clot is also present in the right ventricle. *Stomach.*—Greatly distended (five times its normal size). *Intestines.* (*a*) *Small.*—Ecchymotic spots throughout the mucosa and peritoneum. No ulceration or enlargement of the lymph nodes. (*b*) *Cecum.*—Distended, black, and tarry, and almost in a gangrenous condition throughout. (*c*) *Colon.*—Ecchymotic, congested. and in parts black; not in quite as bad condition as the cecum. *Liver.*—Congested, soft, light in color, and showing parenchymatous degeneration. *Spleen.*—Slightly enlarged, somewhat friable, and showing parenchymatous degeneration. *Kidneys.*—Adherent capsules. Both kidneys are normal in size. The right kidney shows a large infarct in the medulla, about the size of a silver dollar. Both show evidences of parenchymatous nephritis. *Cerebrospinal System.*—The meninges of the brain and cord are ecchymotic, and the blood vessels congested. The brain and cord are otherwise negative. The cerebrospinal fluid is somewhat under pressure but clear. *Lungs.*—On microscopical examination the lungs show the usual changes of lobar pneumonia, and all the organs those of extreme granular and fatty degeneration.

BACTERIOLOGY.

Bacteriological examination of the intracardiac blood, lungs, liver, spleen, kidneys, and cerebrospinal fluid of horse 1 revealed upon smears a Gram-negative bacillus and a Gram-positive, encapsulated, lancet-shaped diplococcus. Cultures were also made at the same time from the same sources and plated on blood serum agar, agar, glucose agar, gelatin, etc. At the end of twenty-four hours a luxuriant growth appeared and two types of colonies could be plainly seen. (1) A pin point colony, round, transparent, moist, and flat, which, microscopically, was finely granular, with a dark center and slightly corrugated, lighter colored peripheral areas. Subcultures from this colony showed a typical pneumococcus. (2) The other colony, which was by far more numerous, was entirely different in type. The colonies were large, varying in size from one to six millimeters, perfectly round, with smooth edges,

elevated at the center, and presented a greyish white mucus-like growth, having a characteristic slimy and semifluid appearance. Morphologically this colony proved to be composed of plump bacilli, varying in length, with rounded ends when singly distributed, as was usually the case. A few diploform and short chains presented square ends. Its average measurements are from 0.5 to 1.5 microns in width and from 0.6 to 5 microns in length, it is extremely motile, has no spores, no metachromatic granules, and no capsule; it is Gram-negative and stains readily with the aniline dyes. Filamentous forms are sometimes seen. In old cultures it occasionally shows a bipolar appearance with an intervening light staining area. When cultivated upon the various media the type of colony produced was the same as the one described above. Upon successive subcultivations, however, the colony lost its mucoid appearance and became smaller in size. When grown upon a sugar medium and in the presence of a good deal of moisture, the original type of colony reappears to a certain extent. It grows well upon the ordinary laboratory media. Upon agar slants the colony varies in size from 0.5 to 1 millimeter, is elevated in the center, translucent, colorless, with round edges and produces no pigment. Upon gelatin it grows well at room temperature without liquifaction (ten days.) On potato medium it grows sparingly, showing a moist, colorless, almost invisible growth. On plain bouillon it produces a diffuse cloudiness, no pellicle, and a heavy greyish white precipitate. It acidifies but does not coagulate milk. It produces acid but no gas on dextrose, lactose, maltose, and mannite. It does not ferment or produce acid on saccharose or dextrin. It produces no indol on Dunham's peptone. Agglutination reactions against typhoid serum were negative. Agglutination reactions with the serum of the sick horses, as well as with those that recovered, were positive; agglutination was still present in a dilution of 1 to 200 six months after recovery. It is pathogenic for mice, rats, rabbits, and guinea pigs. At autopsy the animals showed marked parenchymatous degeneration of all the viscera, and the same organism was isolated. It is not pathogenic for dogs.

I inoculated this organism into a horse brought from the farm and kept in a stable at some distance from the armory. The animal was under the constant observation of Dr. Tiersen.

Twenty-four hours after the intravenous inoculation of a saline emulsion of six agar culture tubes inoculated with the bacillus and incubated for eighteen hours, the horse's temperature rose abruptly to 105° F., and it refused nourishment and exhibited signs of marked weakness and constipation. The temperature for the next three days fluctuated between 103° and 106° F., gradually returning to normal at the end of five days. The cardinal symptoms of the disease, namely, hyperpyrexia, slow pulse, anorexia, weakness, and constipation, were reproduced in the experimental animal. No attempt was made to recover the microörganism from the experimental horse.

THE TREATMENT OF THE DISEASE.

Drs. Pilcher and Bell attempted early in the epidemic to immunize passively the sick horses by transferring the serum of horses that had recovered from the disease. No benefit was obtained from this procedure after repeated attempts, and hence it was abandoned. Various drugs were employed, such as quinine, tincture of nux vomica, digitalis, belladonna, laxatives, intestinal antiseptics, etc., but without success. On January 4, 1912, I employed vaccine therapy. A vaccine composed of 800,000,000 of each species of bacteria, the pneumococcus and the bacillus isolated, was injected subcutaneously in the region of the neck of three sick horses (Nos. 2, 3, and 4). The animals represented three phases of the disease. Horse 2 was in the terminal stage, horse 3 in the intermediate, and horse 4 in the initial stage of the disease. As the animals showed marked improvement at once, it was decided to administer the same dose of vaccine to all the sick horses. About twenty hours after the injection there was in all cases a marked rise in temperature followed by a drop to a point below that at the time of injection. All the symptoms ameliorated, there was a return of appetite and strength, regular bowel movement, and a return of temperature to normal by lysis. The injections were repeated at the end of three days, and rarely more than two inoculations were necessary before the animal was cured. Horse 5 was vaccinated immediately upon the discovery of the disease and in this case the attack was slight.

TABLE I.

The Reaction of Normal Horses after Immunization with Vaccine.

No. of horse.	Normal temperature prior to vaccination.	Temperature 18 hrs. after vaccination.	No. of horse.	Normal temperature prior to vaccination.	Temperature 18 hrs. after vaccination.
1	99 4/5°	102°	38	100 3/5°	103 2/5°
2	100 1/5°	100 2/5°	39	100 2/5°	104 1/5°
3	99 2/5°	100 3/5°	40	100 2/5°	103 4/5°
4	99°	102°	41	100 2/5°	103 2/5°
5	100°	101°	42	100 3/5°	104 1/5°
6	100°	102 2/5°	43	100°	103 1/5°
7	99 2/5°	101°	44	100°	103 2/5°
8	100°	102°	45	100 1/5°	104°
9	100°	102 4/5°	46	100°	103 3/5°
10	102 1/5°		47	100 2/5°	104°
11	100 1/5°	101 1/5°	48	99°	105°
12	100 1/5°	102 1/5°	49	100 2/5°	103 2/5°
13	100 1/5	99 4/5°	50	100°	103 4/5°
14	100°	103 4/5°	51	99 4/5°	101 4/5°
15	100 1/5°	102 3/5°	52	100 2/5°	103 3/5°
16	100°	100 4/5°	53	100 1/5°	104 4/5°
17	99 4/5°	101 4/5°	54	100°	103°
18	100 4/5°	103°	55	100°	102 4/5°
19	99 3/5°	100 3/5°	56	100 3/5°	104°
20	100 2/5°	101°	57	100°	102°
21	100°	101 3/5°	58	100°	103 2/5°
22	99 1/5°	101 2/5°	59	99 4/5°	101 2/5°
23	100 2/5°		60	99 2/5°	100 4/5°
24	99 4/5°	102°	61	99 3/5°	102 4/5°
25	100°	102 2/5°	62	100°	101 2/5°
26	99 3/5°	103°	63	100 2/5°	101°
27	99 4/5°	102°	64	99 2/5°	100 2/5°
28	100°	102 3/5°	65	99 4/5°	101 3/5°
29	100°	102 1/5°	66	99 3/5°	102 1/5°
30	100°	100°	67	99°	101 3/5°
31	100 2/5°	102 2/5°	68	100°	101 1/5°
32	100°	102 2/5°	69	100 1/5°	102 2/5°
33	99 4/5°	102°	70	100°	104 2/5°
34	100°	102 3/5°	71	100 2/5°	102 3/5°
35	100 1/5°	101°	72	100 3/5°	103 2/5°
36	99°	103°	73	99 4/5°	103°
37	100°	104 2/5°	74	99 3/5°	103 4/5°

Six normal horses of different types were then vaccinated with 50,000,000 of each organism (bacillus and pneumococcus) in order to immunize them against the disease. They reacted in various ways, some being but slightly, if at all, affected. One reacted violently, the temperature rising to 105° F. ten hours after the injection, and presented all the symptoms of the accustomed disease, subsiding gradually to normal at the end of four to five days. Most of the horses reacted moderately. At the end of ten to twenty hours,

the temperature rose to 101° to 102° F., the pulse was but slightly affected, and the horse would refuse food and show signs of temporary weakness; but at the end of twenty-four to forty-eight hours there was a return to normal. After this the remaining normal horses were inoculated and reacted similarly to the cases cited (table I).

The immunity conferred by vaccination apparently does not last more than one year; for the next year a slight epidemic of the same disease broke out among the horses that had been immunized the year before. The same procedure was followed, that of vaccinating the affected as well as the unaffected horses, and the epidemic was promptly eradicated, not a single case proving fatal. The immunity lasts much longer if three inoculations are given at intervals of three days. None of the horses died that were treated with vaccines, in spite of the fact that one was *in extremis*. Furthermore, none of the immunized horses developed the disease.

In conclusion I wish to express my indebtedness to Colonel Charles I. DeBevoise, and to the veterinarian of the squadron, Dr. Walter L. Bell.

EXPERIMENTS ON THE TRANSMISSION OF SCARLET FEVER TO THE LOWER MONKEYS.*

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The subject of the experimental production of scarlet fever in the monkey is at present under discussion. Indeed, it was partly because of the discordant statements in the literature that we were led to undertake a detailed study of the question, as the successful transmission to the lower animals of an otherwise refractory disease has often been followed by an important increase in the fundamental knowledge of the affection. The etiology of scarlet fever is still unestablished and there exists as yet no specific mode of treatment. Furthermore, it is uncertain whether a streptococcus bears a direct or only an indirect relation to the disease, although it is known to be active in causing secondary or concomitant infections, either septicemic or suppurative in nature.

Whatever the causative agent may be, there is no doubt of the inoculability of scarlet fever upon healthy human beings. Proof of this is found in the history of obstetrical practice of a generation ago and in the epidemical nature of the disease. The important and often fatal part played by the streptococcus in the former instance is well known. Stickler's observations tend, moreover, to show that the germ of scarlet fever is able to resist 0.6 per cent. carbolic acid, but the writer fails to state for how long a period. Stickler also supports the view, now generally accepted, that the virus is contained in the secretions of the mucous membrane of the nose and throat.

The results with animals are less convincing. The ordinary laboratory animals appear to be wholly refractory to inoculation with materials carrying the virus. There is general agreement re-

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garding this point. The question turns upon the susceptibility to inoculation of monkeys, both higher and lower.

In 1904 Grünbaum produced doubtful symptoms in a chimpanzee which he clad in the nightshirt of a scarlet fever patient. In addition to this, the animal's throat was thoroughly rubbed with swabbings from the pharynx of a case of scarlet fever. In 1911 Hektoen and Weaver failed to obtain any result from feeding *Macacus rhesus* monkeys upon milk contaminated with swabbings from the throats of scarlet fever patients. The present revival of interest dates from Cantacuzène's announcement that infection of lower monkeys (*Macacus rhesus, Macacus sinensis, Cercopithecus cephus, Cercopithecus griseoviridis*) was accomplished by inoculating them with blood, pericardial effusion, and emulsion of lymph nodes taken from cases of scarlet fever. Bernhardt soon followed with an account of the successful subcutaneous inoculation of a *Macacus rhesus* with scrapings from the tongue, swabbings from the throat, and serum from a bleb of scarlet fever patients, and also from merely swabbing the throat with infected scrapings from the tongue. In a second report he states that a Berkefeld filtrate prepared from lymph glands produced identical symptoms on injection into *Macacus rhesus* monkeys. From this he concludes that the virus is filterable. We shall return to a consideration of the symptoms described by these authors as evidence of successful infection. The most recent publication on the subject is that of Landsteiner, Levaditi, and Prasek who report the appearance of suggestive but unconvincing symptoms as the result of inoculating a chimpanzee. They proceeded as follows: Three chimpanzees were used. The first animal's throat was rubbed well with a deposit from the tonsils of a child with scarlet fever. Three days later the animal received subcutaneously 75 c.c. of defibrinated blood from another case. Two days later the symptoms began to appear. Death occurred in ten days. The second chimpanzee was inoculated repeatedly as follows:

June 18. 20 c.c. of defibrinated blood, subcutaneously; tonsils swabbed with the deposit from the throat of the patient.

June 19. 10 c.c. of defibrinated blood, subcutaneously; throat swabbing repeated.

June 21. 5 c.c. of emulsion of cervical glands from a fatal case, subcutaneously (material glycerinated).

June 23. 5 c.c. of blood, subcutaneously; tonsils swabbed. Death in six days.

The third chimpanzee was inoculated less intensely with tonsillar deposits and blood, in the throat, subcutaneously, and intraperitoneally. The animal recovered in six days, after a course with angina, false membrane, rise of temperature, and diarrhea. At autopsy a streptococcus was isolated in pure culture from the blood of the two fatal cases. A subsequent protocol indicates that one attack, such as that described for chimpanzee 3, does not confer immunity.

The symptoms upon which all the observers base their conclusions are varied and indefinite. All describe skin manifestations, but each differs markedly from the others. Thus, of the two sets of observers who used chimpanzees, Grünbaum speaks of a "doubtful

roseola not having a scarlatiniform appearance," while Landsteiner, Levaditi, and Prasek describe an "exanthem of tiny red spots and confluent areas." Cantacuzène and Bernhardt, working with the lower monkeys, present equally diverse descriptions of the rash. The former writes of "a uniform purple eruption on forehead, face and sometimes on the arms," which paled in thirty-six hours; whereas Bernhardt reports simply a "blotchy redness." Here is a striking dissimilarity in the description of a symptom which, from our present knowledge of the disease, must be looked upon as one of the most important. The chimpanzees are reported by both sets of observers to have shown a white exudate on the tonsils associated with an intense angina. Grünbaum does not mention the appearance of the tongue, though Landsteiner and his associates report that the tongues of their animals were red with large follicles. No throat involvement in the lower monkeys is mentioned by Cantacuzène or Bernhardt, and the latter alone mentions that his *Macacus rhesus* showed the typical coated raspberry tongue. On the question of desquamation Cantacuzène and Bernhardt agree that a large flaked scaling occurred, especially of the face and head, but Grünbaum and Landsteiner do not mention it. All the authors report rises of temperature to 40° and 41° C.

The symptoms described by these writers as evidences of scarlet fever in the monkey are not convincing (table I). They consist of variable moderate rises of temperature, irregular erythemas, and desquamation. Throat changes are described only by those who used chimpanzees. The question arises, therefore, whether any other test can be invoked to determine whether a specific infection has been accomplished. If we admit for the moment that the outspoken symptoms following some of the inoculations are evidences of a scarlatinal infection, it might be held that an immunity to reinoculation may be produced that furnishes, in doubtful instances, a means of determining whether a previous specific infection existed or not. In the only instance in which this test was applied, however, no immunity was demonstrated (Landsteiner, Levaditi, and Prasek).

But this is debatable ground. Analogies exist between the indefinite instances of so called specific infection in the monkey and

TABLE I.

	Temperature.	Rash.	Desquamation.	Throat.	Tongue.	Glands.
Grünbaum	39° C.	Doubtful roseola not having scarlatiniform appearance.		White spots becoming an exudate on both tonsils. Streptococcus isolated from throat.		
Cantacuzène	Rise to 40° C. Fluctuated for 23 days between 40° and 41° C.	Uniform purple eruption on forehead, face, and sometimes on forearms. Paled at end of 36 hours.	At end of 36 hours, large flaked desquamation of face. Small scales on back, head, neck, and extremities.			General adenopathy persisting for a long time.
Bernhardt	Normal, 38° C. Highest 40° C. Gradual fall.	Blotchy redness on face, shoulders, and neck.	Scaling; large flakes, including palms and soles.		Coated raspberry.	General swelling.
Landsteiner, Levaditi, and Prasek	38° to 40° C.	Exanthem of tiny red spots and confluent areas on neck, head, and extremities.		Intense angina of tonsils, pillars, and mucous membrane. White deposit on tonsils.	Red with large follicles.	

mild and almost unrecognizable cases of scarlet fever in man. However, in an experimental investigation of this nature only definite evidences of illness can be admitted as evidences of infection.

The quest is complicated by another factor. The causative agent of scarlet fever is still undiscovered. Its detection cannot, therefore, be utilized as evidence of infection. On the other hand, the streptococcus is a constant attendant of the scarlatinal infection; it exists not only in the nasopharynx, but often in the blood also. This microörganism may produce its own effects when materials containing it or its growth products are injected. To distinguish between the effects of the streptococcus and of the unknown agent of scarlet fever offers, therefore, another theoretical difficulty.

These considerations led us to employ merely the most prominent

symptoms in the monkey as evidences of possible infection, and no single sign, such as local erythema, slight rise in temperature, vomiting, or desquamation. Even when two or three signs coincided, they were taken to be suggestive rather than demonstrative. Viewed in this way, the import of the effects upon lower monkeys of the inoculation of materials from scarlet fever patients does not confirm the observations of Cantacuzène, Bernhardt, and others.

THE SOURCE AND NATURE OF THE INOCULATING MATERIALS EMPLOYED.

The materials employed for inoculation were derived from patients in the early stages of scarlet fever. This was rendered possible by the coöperation of the diagnosticians of the Department of Health. Several cases were received in the hospital within twelve hours, and many within twenty-four to thirty-six hours after the first appearance of the rash.

The materials used may be divided into three classes as follows: (1) artificially sterilized; (2) fresh unsterile; (3) fresh sterile.

The artificially sterilized material consisted of sputum treated with carbolic acid, so that the mixture contained 0.5 per cent. of the disinfectant. This combination was shaken in a machine for one half to one hour, then put in the thermostat for a few hours and shaken occasionally by hand. Sometimes in twenty-four, and usually in thirty-six hours, cultures from the mixture on glucose agar were sterile. This procedure has been shown by Flexner to destroy the bacteria contaminating poliomyelitic virus, without injuring the virus. After several experiments it became evident that the monkeys were highly resistant to infection by the microörganisms in the nasopharyngeal and ear secretions of human beings. Consequently the carbolized material was soon abandoned and fresh substances were transferred directly from patient to animal.

The fresh unsterile material consisted of (*a*) whole sputum shaken up with enough salt solution to render it evenly emulsified, (*b*) tongue and throat scrapings similarly shaken, (*c*) absorbent cotton tampons from the nasopharynx, (*d*) finely chopped tonsils, (*e*) washings from extirpated tonsils, (*f*) discharge from the ear.

This unsterile material was injected subcutaneously, submucously, and intraperitoneally without serious effect. Indeed, with the exception of sputum from a single individual, a general reaction was never observed, and in only two or three instances did local abscesses develop. Cultures from the pus of the abscesses almost invariably showed *Staphylococcus aureus,* and in no instance was the predominating organism of the injection mass recovered.

The third class of scarlatinous material was composed of (*a*) urine obtained under sterile precautions and found sterile by culture; (*b*) whole blood collected from a vein in paraffin-coated iced tubes, and injected, before clotting, intravenously into a monkey; (*c*) defibrinated blood; (*d*) sputum filtrates (Berkefeld); (*e*) filtrates of broth cultures of streptococcus from scarlatinous throats; (*f*) blood from scarlet fever cases mixed with ascitic broth and incubated at 37° C.

OBSERVATION OF THE INOCULATED MONKEYS.

The routine observation of the inoculated monkeys consisted in temperature readings twice daily, frequent blood counts, and physical examinations. Control observations were made on several normal monkeys over a period of about ten days before the regular inoculation series was begun. As a result of these preliminary findings, normal monkey temperature curves were considered unsatisfactory standards by which to measure pathological variations. Indeed we attached little significance to the irregularities of temperature which the animals showed, even when the readings were as high as 41° C. In several instances after a few days of rather high, irregular temperature the curve fell and continued almost level (Ringtail 2, *Cercocebus fuliginosus* 3). Occasionally, also, a temperature curve might show a sudden sharp rise for which no explanatory physical sign or treatment could be determined.

In contradistinction to the numerous irregularities in the temperature of stock animals were the remarkably even curves of many inoculated monkeys. For example, *Macacus rhesus* monkeys 10 and 12 received respectively five and four inoculations, yet their temperature curves were almost undisturbed. Still more striking

is the case of *Cynocephalus babuin* 16, who developed a purulent panophthalmitis, necessitating enucleation of the eyeball; yet, although his temperature was irregular, at no time did it rise above 40° C. Another paradoxical group was formed by the animals that began with a high temperature and whose curve sank gradually but steadily after inoculation (*Macacus rhesus* monkeys 29 and 30, and *Macacus nemestrinus* 20). In the case of *Macacus nemestrinus* 20 the lowest part of the temperature curve corresponded with the time when suppuration of the thighs existed. *Macacus rhesus* 25, on the other hand, displayed a distinct rise of temperature when abscesses formed in the groin.

These examples show how uncertain a criterion the temperature of the monkeys is. The blood counts were still more unsatisfactory and were given up early in the work.

A thorough physical examination of the animals was made every morning, and suspicious signs were watched during the day. It soon became evident that the superficial lymph nodes would be of little value, because in almost every animal they were enlarged, especially the axillary nodes. This was a constant finding. Furthermore, many of the animals, especially the *Macacus rhesus* monkeys, developed from time to time purplish red erythemas of varying intensity on the face and neck. In the case of one female with an unusually brilliant color about the buttocks, these erythematous blotches appeared in association with an intensification of the buttock redness. At the same time there was a bloody, mucous, vaginal discharge. The *Macacus nemestrinus* monkeys were much paler and in no case showed any redness. The *Macacus rhesus* monkeys likewise exhibited almost constantly a fine, bran-like desquamation, seen more especially over the neck, shoulders, and chest. It is difficult to see the tonsils in monkeys, for they are small and low down, and the faucial ring is narrow. In other respects the throat is easy to observe and in our series was always found to be rather pale with a slightly livid tinge. The tongue of monkeys is usually clean, though at times an irregularly light whitish coat may be seen. The papillæ tend to be prominent and pink.

EXPERIMENTAL OBSERVATIONS.

The experiments were varied as much as possible, both with respect to the character of material and the site and method of inoculation, on the one hand, and the conditions bearing upon the susceptibility of the animals, on the other.

The routes of introduction were through the mucous membranes, skin, stomach, joint cavities, blood stream, peritoneum, and brain. It seemed possible that repeated inoculations at three week intervals over a long period might bring about a state of hypersensitiveness and so lead to infection. Consequently several monkeys were treated in that manner. The animals stood the inoculation well and showed no reaction of any kind. In this series most of the inoculations were made by swabbing the scarified mucosa with infected material, and by submucous injections in the throat and nose with a hypodermic needle.

With the view of adapting a virus to an unusual host, two series of experiments were undertaken, in which infected material was injected subcutaneously in a region whose lymphatic apparatus was easily accessible for removal. The groin was used in each series, but the inoculation material differed. Series A received subcutaneously the supernatant fluid from an emulsion of fresh tonsils from scarlet fever patients, whereas series B was given an emulsion made from tongue scrapings. It is interesting to note that after the first passage the inguinal lymph nodes were sterile, although in some cases enlarged.

The first three animals of series A were treated as described above and the inguinal nodes were removed and an emulsion made from them was reinjected into another monkey. The last three animals of this series were treated in a similar way, except the final one, which received the emulsion of inguinal nodes partly in the brain and partly in the peritoneum (*Macacus rhesus* 28). Series B was a repetition of Bernhardt's method. Both series were negative.

Clinical experience suggests that scarlet fever and measles are not infrequently associated. This may depend purely upon coincidence, but the fact suggested to us the possibility that one disease might predispose to the other. Consequently, encouraged by the

successful reports by Anderson and Goldberger of the transmission of measles to *Macacus rhesus* monkeys we attempted to produce this disease in an animal which was to be inoculated later with scarlatinous material. Neither disease developed in the two animals injected.

The high degree of resistance shown by these animals led us to try two other modes of infection. The first was designed to overwhelm the animals with large, rapidly repeated inoculations of the infected material, and the second to employ methods which are known to lower the resistance of animals to infection. The following protocol illustrates the methods used.

A healthy *Macacus rhesus* monkey, giving a negative von Pirquet tuberculin test, was inoculated as follows:

February 23. 3 c.c. of defibrinated blood from a patient one day ill, intracerebrally; 25 c.c. of defibrinated blood from a patient one day ill, intraperitoneally.

February 24. 20 c.c. of an emulsion of inguinal nodes from *Macacus rhesus* 24 (previously inoculated), subcutaneously in the right groin and thigh.

February 25. 43 c.c., intraperitoneally, of a mixture made of 30 c.c. of fresh blood from a twenty-four hour old case of scarlet fever, incubated in 75 c.c. of ascitic broth at 37° C. for forty-five hours.

February 27. The throat, pharynx, and tongue were scarified and swabbed with fresh throat swabbings of a five day old case of scarlet fever.

March 2. 95 c.c., intraperitoneally, of a Berkefeld filtrate of the following materials: sodium chloride emulsion of fresh sputum of a five day old case of scarlet fever plus two broth flask cultures of streptococcus from the throat of a scarlet fever patient.

Notwithstanding this amount of inoculation, the animal showed no rise of temperature above 40° C. and no signs of illness or discomfort.

To diminish the resistance of the animals, the states of exsanguination and of anaphylactic shock were produced. In the first instance the animal was bled until severe symptoms of hemorrhage appeared and then a saline infusion was given. Following this, 50 c.c. of fresh whole blood were transferred directly from the vein of a twenty-four hour old case of scarlet fever into the circulation of the monkey. On the next day the monkey's throat and pharynx were swabbed with material from the throat of the same case that had supplied the blood. No symptoms developed. The last monkey treated was given a mild anaphylactic shock by injection of horse serum at proper intervals. On the day before the shock the animal received intracerebrally 2 c.c. of defibrinated blood from a forty-eight hour old case of scarlet fever. Four hours after the shock 3.5 c.c. of the same defibrinated blood were inoculated intracerebrally and 15 c.c. intraperitoneally. The pharynx was also swabbed with throat swabbings from the same case. No symptoms developed.

In the case of the exsanguinated monkey it is conceivable that the saline infusion acted as a diluent and thereby lowered still further the defensive mechanism of the body, already partially depleted of blood. Just how much the anaphylactic shock given to monkey 29 lowered its resistance to infection is problematical, especially in view of the fact that the blood used for inoculation of the brain and peritoneum contained streptococci. The animal developed no symptoms.

CONCLUSIONS.

1. The reported successful transfer of scarlet fever to both higher and lower monkeys is not definitely established.

2. In the course of the experiments here reported, the infectious agent can be assumed to have been carried over to the monkeys. The failure to cause infection probably proceeds from the insusceptibility of the monkeys employed, or to the manner of introducing the agent.

3. The temperature curve and leucocyte count of monkeys are unsatisfactory criteria for the diagnosis of disease in those animals.

4. Monkeys frequently have transient blotchy, erythematous eruptions on the face and neck, and almost always a bran-like desquamation.

5. Monkeys are highly resistant to infection with microörganisms from human beings.

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EXPERIMENTAL PULMONARY TUBERCULOSIS IN THE DOG.

The Effect of Large Amounts of Tubercle Bacilli of Bovine Type Introduced Directly into the Lungs by Way of the Air Passages.*

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Plate 101.

This study was undertaken because of the apparent desirability of having at our command methods of reproducing as nearly as possible in experiment the conditions that prevail in chronic pulmonary tuberculosis.

Chronic pulmonary tuberculosis presents a complex picture, the result of the interaction of a number of factors. Important among them are, first, the severity of the infection as represented by the number and possibly also the virulence of the bacilli gaining entrance to the lungs; second, the resistance of the individual to infection as manifested in the reaction to the primary contagion and as modified during the course of the disease either by the specific reactions of immunity and hypersusceptibility or by physiological changes not directly associated with the disease; and third, the complication with other diseases and infections, particularly the direct secondary infection of the affected lung tissue.

Thus far we have been no more successful than our predecessors in so combining the various factors under controlled conditions as to reproduce chronic pulmonary tuberculosis with all its characteristic features. Nevertheless these factors, or some of them, are of decisive influence on the result of any experimental production of

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tuberculosis, and the fact that we have failed so far to reproduce in characteristic form the complete picture cannot lead to the conclusion that it would not be possible to do so by altering the conditions of the experiment.

We have, in the course of work to be described, reproduced almost exactly a certain rare form of tuberculosis of the lungs, acute tuberculous lobar pneumonia, and on this account we feel that the experiments are worthy of brief description. This is especially so in view of the fact that we have closely followed the technique by the use of which Lamar and Meltzer (1) succeeded in producing an acute lobar pneumonia with the pneumococcus, and with which Wollstein and Meltzer (2) later produced lobular pneumonia with the streptococcus. Our results may lend further significance to their work.

Assuming that the final result of our experiments would depend in large measure on the balance between the resistance of the dog, commonly classed as highly resistant, and the virulence of the culture, we selected for our work a culture from human source, but of bovine type, known to be virulent for guinea pigs, rabbits, and cattle. Actively growing cultures between ten days and two weeks old were used. The culture was thoroughly emulsified in salt solution by the use of an agate mortar. The small clumps remaining were disregarded. Ten cubic centimeters of salt solution were used for each culture tube, the culture mass weighing approximately 100 milligrams. In most of the experiments ten cubic centimeters of the emulsion were administered. In a few instances one cubic centimeter was given further diluted with nine cubic centimeters of salt solution.

The mode of administration was as follows: The dog, etherized to the point of complete relaxation, was laid on its back. The mouth was opened and the epiglottis brought forward until the orifice of the larynx was plainly visible. A medium sized soft catheter was then inserted into the larynx and pushed on through the trachea until it was judged that the opening was below the bifurcation. The catheter was then quickly attached to the syringe containing the emulsion and this was injected slowly. The catheter was quickly removed and the animal was allowed to turn on its right side. During removal from the table to the floor the animal was kept on its right side with the head somewhat higher than the tail. Recovery from the procedure was always prompt and unaccompanied by cough or other symptoms of distress.

It may be said here that at autopsy it was found that in all instances the inoculation had been made chiefly into the right caudal lobe. In most instances there was a considerable diffuse consolidation of the right middle lobe and some focal involvement of other lobes, particularly the left caudal lobe.

Following the injection the animals show no marked changes for five days. In from six to nine days they begin to cough. The cough, at first occasional, gradually becomes frequent and severe. At about the same time the animal begins to lose weight and condition. The loss of weight is progressive and at the time of death it amounted frequently to one third, and has sometimes been nearly one half of the original weight.

Percussion and auscultation show a well defined progressive consolidation beginning with indefinite signs about the time of the cough and becoming pronounced a few days later. It is perfectly possible to follow the course of the lung lesion by clinical methods. but the conditions do not permit of the accurate observations attained by Opie (3) in his study of the pleural exudates in the dog.

The temperature of some animals has been taken throughout the course of the infection and in others at irregular intervals. No fever has at any time been noted.

In only one case has there been any complication. One animal in a severe fit of coughing caused a pin point rupture in a healthy portion of the lung, developing a pneumothorax. This was at first unilateral but became bilateral a few hours later and the animal died. Most of the animals in the series died or were killed when moribund. A small number were killed in the earlier periods of the experiment in order to follow the course of development of the lesion.

POST-MORTEM EXAMINATIONS.

On gross examination at autopsy, there was always found a consolidation of the lung varying in extent and character with the length of time the animal had survived the inoculation and the amount of culture administered.

In the usual case the consolidation was found most pronounced in the right caudal lobe. Where the larger amounts of culture were

given, this lobe was completely or almost completely pneumonic. The consolidation varied somewhat in color and consistency. In the earlier cases (before the fourteenth day) whether the animal died or was killed the color was reddish gray. Later it was gray, yellowish gray, yellow, and in one late instance almost white. The tissue was firm, quite dry, and very little material could be scraped off with light pressure on the knife. In the later stages there was a tendency for the tissue to soften in spots, and in those cases examined between the seventeenth and the twenty-fourth days the softened matter had frequently been discharged through a bronchus, leaving a cavity.

The cavities were variable in distribution and shape. Sometimes they were large and single. In other instances they were small, irregular, and connected to form ramifying excavations throughout the lobe. In the formation of the cavities the blood vessels were frequently left exposed. No instance of hemorrhage from this source was noted. There was no tendency towards the formation of a membranous wall in connection with the excavations. Figures 1 and 2 illustrate the types of cavities found.

In the animals examined more than three weeks after infection there was always a miliary tuberculosis of the unconsolidated portion of the lungs, the spleen, the kidneys, and especially of the liver.

The pleuræ over the consolidated areas were usually somewhat dull. When the cavities approached the pleural surface the pleura was always definitely thickened. In no instance was there a definite pleural exudate.

The earliest microscopic examinations made have been of animals dying or killed ten days after inoculation. The process at this time is entirely exudative in character. The character of the exudation is extremely variable when the alveoli are considered. Alveoli showing the various types of exudation are, however, so intermingled as to make it impossible to consider that the exudation has first affected particular regions and spread to others. The contents of single alveoli are usually predominantly of one order. Many alveoli are filled with fibrin and show very few cells. Others contain predominantly red blood corpuscles, while still others show chiefly leucocytes. Of the alveoli showing leucocytes many have

chiefly polymorphonuclear cells, while others show mostly endothelial phagocytic cells. Cells of endothelial type are also found in large numbers in the smaller blood vessels, particularly in the dilated capillaries of some of the alveolar walls. Cells of this type both in the vessels and in the alveoli are actively proliferating, as evidenced by the presence of numerous karyokinetic figures.

At this period the contents of single alveoli and in a few instances of small groups of alveoli begin to show necrosis. Bacilli are most numerous in the necrotic areas. Small groups of bacilli are found in other areas. While the bacilli are sufficiently numerous to be demonstrated with ease there are probably less at this stage than the number originally introduced.

From the fourteenth to the twentieth day necrosis and caseation become more extensive. The necrosis, as indicated above, seems always to begin in the exudate. When it becomes well marked the adjoining fixed tissues become involved. Cavity formation may occur when the fixed tissues are involved in the necrosis. In this type of lesion, at least, cavity formation occurs by a necrosis extending into the bronchus from the surrounding exudate. No instance of a primarily ulcerated bronchial mucosa has been observed. In this period also the visceral pleura becomes thickened. The bacilli increase rapidly in number and at the end of the third week are extremely abundant. The order of the changes described has always been observed in this series of experiments but the division into periods is arbitrary, as the process varies in its rapidity of progress in different animals. One animal, for example, dying twenty-two days after inoculation, showed the microscopic picture described for those dying or killed about the tenth day.

The cavities that are formed correspond in some particulars to the cavity of chronic tuberculosis. Particularly the blood vessels show a considerable resistance to ulceration and remain intact, exposed in the cavity. In other particulars correspondence is entirely lacking. The wall of the cavity is composed of a necrotic exudate which differs very little microscopically from the detached contents. Macroscopically the exudate in the cavity is moist while that of the wall is relatively dry.

After the twentieth day in most cases there are some evidences of

a reparative process. The special stains for connective tissue begin to bring out foci of its formation. The arrangement of the newly formed connective tissue is, however, without order,—especially it has no definite relation to the walls of the cavities. As a reparative process the connective tissue formation is entirely inefficient throughout the period of these experiments.

BACTERIOLOGY.

The rapid increase in the number of tubercle bacilli in the later periods of the experiment has been noted. Cultures have been made and sections examined for other bacteria. As a rule, none have been found. It is especially important that even in most instances of open cavity formation the cavities contained only the tubercle bacillus. In the few instances noted where secondary infection was observed it could not be shown that the character of the process had been in any way influenced thereby.

DISCUSSION AND CONCLUSIONS.

It is obvious, as stated in the beginning, that these experiments have failed to reproduce a chronic pulmonary tuberculosis. Acute pneumonic processes of tubercular origin are, however, not infrequent as a complicating or terminal process in pulmonary phthisis, and there have been several cases described in which individuals have died with the symptom complex of acute lobar pneumonia, in which it developed at post-mortem examination that the disease was tuberculosis. Our experiments throw some light on debated questions in connection with these acute pneumonic forms of phthisis.

Orth (4) argues for a distinctively exudative form of tuberculosis as contrasted with the productive form which leads to tubercle formation. He lays especial stress in this connection on the presence of a fibrinous exudate in alveoli with normal walls. He notes that red blood corpuscles and white cells are frequently associated with the fibrin. The white cells usually predominate. These fibrinous masses usually contain tubercle bacilli and they tend to become caseous from the center. Associated alveoli are found, filled with endothelial or epithelial cells. Orth believes, in opposition to von Buhl and Baumgarten, that the evidence that these are desquamated epithelial cells is scanty and thinks that they are more apt, in part at least, to come through the blood. Orth does not speak of cavity formation in this connection but considers that this process in its essentials occurs in connection with ordinary chronic tuberculosis and that the

exudative process is the destructive one. Etiologically considered, the process is probably due to the sudden access of a large number of bacilli.

Orth speaks of all attempts to reproduce the lesion as having failed or as being imperfect in that fibrinous exudation was not developed and productive lesions usually resulted.

Tendeloo (5) describes a case of acute tuberculous lobar pneumonia and discusses a case previously reported by Fraenkel and Troje (7). Tendeloo believed that his case arose by the discharge of tuberculous material from an old softened focus into a vein. He explains the formation of predominantly exudative lesions in the lungs and other organs, instead of the usual type of miliary tubercle, as being possibly due to the associated presence of necrotizing substances derived from the old focus. Fraenkel and Troje believed their case to be of bronchogenic origin.

In our experiments we believe that we have effectively reproduced this exudative type of tuberculosis of the lungs. This type of lesion may, in the light of our experiments, result from the sudden bronchogenic invasion of large numbers of virulent tubercle bacilli in a relatively resistant animal.

The large mononuclear cells so frequently encountered in the exudate seem to be brought there chiefly from outside.[1] As Orth found when studying human material, it is difficult here also, to exclude the possibility that similar cells may result from the desquamation of alveolar epithelium.

The tubercle bacillus alone, without the aid of secondary infection, is competent to produce a type of lesion which leads to necrosis of the bronchi, and by discharge of the necrotic material to cavity formation. As judged by our experiments the bronchi are less resistant to involvement in the necrosis than are the blood vessels or pleural surfaces.

The fact that by the method of bronchial insufflation of Lamar and Meltzer a characteristic tuberculous pneumonia may be produced makes it probable that the lobar pneumonias generally are of bronchogenic origin.

[1] Since this study was completed, Evans, Winternitz, and Bowman (6), by the application of vital staining methods, have shown that certain cells which are of the same general type morphologically but which are probably of tissue origin (the histogenic wandering cell or pyrrol cell of Goldman) are active in the early stages of tubercle formation. As this work is still in progress, we have not attempted to apply these methods to the determination of the type of these cells. On general morphological grounds the mononuclear cells of this exudate resemble the large mononuclear cells of the blood and lymphatic organs more than the tissue cells with which they are concerned.

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EXPLANATION OF PLATE 101.

FIG. 1. Dog 31, twenty days after inoculation. Cavity showing communications with a bronchus and exposed blood vessels.

FIG. 2. Dog 15, twenty-four days after inoculation. Ramifying connecting cavity formation.

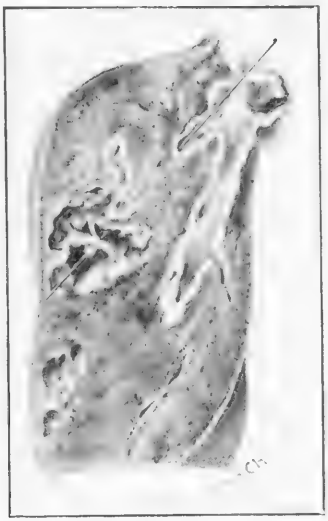

FIG. 1.

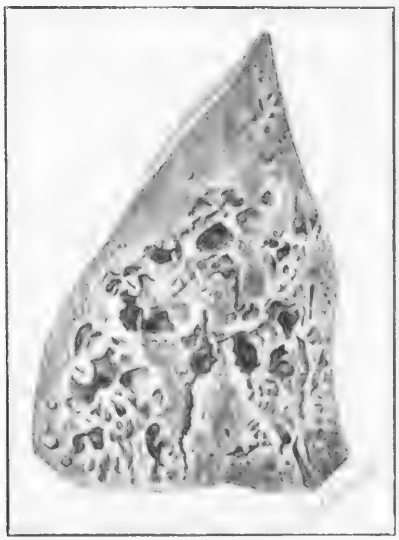

FIG. 2.

(Lewis and Montgomery: Pulmonary Tuberculosis in the Dog.)

THE ANTIGENIC PROPERTIES OF GLOBIN CASEINATE.*

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In previous articles we have considered the antigenic properties of the split products of casein (1), of a synthetic paranuclein (2), and of protamin caseinate (3). The main purpose of these studies has been to throw further light on the nature of biological specificity. As we have previously expressed ourselves, "One of the most promising methods of attack . . . would seem to lie in the search for newly formed antigenic properties in proteins compounded either of non-antigenic proteins, or of an antigenic with a non-antigenic protein." Our experiments in this direction hitherto have for the most part led to negative results. A combination of the amino acids of casein, in themselves non-antigenic, in the proportion in which they occur in casein, failed to produce an antibody. A compound of protamin (salmin), which is non-antigenic, with casein gave rise to an antibody for casein only. It is true that in our comparative studies of split paranuclein with synthesized paranuclein (2) we were apparently able to prove the genesis of antigenic properties in a protein (paranuclein) synthesized from non-antigenic proteoses (products of peptic digestion of casein) by the reversible action of pepsin.

Our experiments have incidentally thrown additional light on the relation that exists between the toxicity or non-toxicity of a protein and its antigenic properties, a relation that has been of particular importance in explaining anaphylaxis. In a general way it seems to be true from our own studies and from those of other investigators that split products of a non-toxic protein, hydrolyzed to

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the point of becoming toxic, lose their antigenic properties. This is not a matter, however, that we are prepared to discuss at the present time.

The present study deals with the antigenic properties of our second compound of casein with a non-antigenic protein. Robertson (4) has described the preparation and properties of the globin and globin caseinate that we have used. As in our previous studies, antigenic properties were sought for, first, by the reaction of anaphylaxis, and, secondly, by the test for fixation antibodies in the sera of animals that had been repeatedly injected with one or the other of the substances under consideration.

ANAPHYLAXIS EXPERIMENTS.

Guinea pigs, weighing from 300 to 400 gm., were given subcutaneous injections of 1 c.c. of a 1 per cent. solution of globin (in N/40 hydrochloric acid) or of globin caseinate (in N/40 potassium hydrate), and after a three weeks' interval were injected with one or the other of the substances in the doses indicated and with the results noted in table I.

The anaphylaxis experiments show the toxicity of globin for normal animals. Globin apparently causes symptoms and lesions in the guinea pig in all respects similar to anaphylactic intoxication by a non-toxic protein-like horse serum, or a substance like peptone. Globin, however, is not antigenic, as determined by its property to sensitize for an increased intoxication by itself; guinea pigs that have received an initial dose of globin react no more violently than normal animals to a subsequent dose of the substance (compare guinea pigs 128 and 139, 129 and 141, and 130 and 140).

Globin caseinate sensitized slightly to itself, more markedly for casein, and not at all for globin. Globin also fails to render anti-anaphylactic an animal sensitized to globin caseinate (133 and 134). In other words, the casein alone seems to function in anaphylactic sensitization and intoxication. These experiments, therefore, throw no further light on specificity.

As will be seen, more definite and instructive results were obtained in fixation experiments with the sera of immunized rabbits.

TABLE I.

No. of guinea pig.	Initial injection, subcutaneously.	Injection after three weeks.	Symptoms.
128	1 c.c. of 1 per cent. globin	4 c.c. of 1 per cent. globin intraperitoneally	Irritation and prostration; recovered.
129	1 c.c. of 1 per cent. globin	1 c.c. of 1 per cent. globin intravenously	Dead in 2 min.; emphysema; hemorrhages into lungs and pericardium.
130	1 c.c. of 1 per cent. globin	1 c.c. of 0.2 per cent. globin intravenously	Marked symptoms; recovered.
131	1 c.c. of 1 per cent. globin	4 c.c. of 1 per cent. casein intraperitoneally	No symptoms.
132	1 c.c. of 1 per cent. globin	1.25 c.c. of globin caseinate intravenously	No symptoms.
133	1 c.c. of 1 per cent. globin caseinate	4 c.c. of 1 per cent. globin intraperitoneally. 24 hrs. later 1 c.c. of 1 per cent. globin caseinate intravenously	Irritative and prostrated symptoms; recovered. Doubtful symptoms.
134	1 c.c. of 1 per cent. globin caseinate	1 c.c. of 0.2 per cent. globin intravenously. 24 hours later 4 c.c. of 1 per cent. casein intraperitoneally	Marked symptoms like Nos. 130 and 140. Slight symptoms.
135	1 c.c. of 1 per cent. globin caseinate	1 c.c. of 1 per cent. globin caseinate intravenously. 24 hours later 4 c.c. of 1 per cent. casein intraperitoneally	Doubtful symptoms. Doubtful symptoms.
136	1 c.c. of 1 per cent. globin caseinate	1.25 c.c. of globin caseinate intravenously	Slight symptoms; killed in 1 hr.; gastric hemorrhages.
137	1 c.c. of 1 per cent. globin caseinate	4 c.c. of 1 per cent. casein intraperitoneally	Marked symptoms.
138	Control	1.25 c.c. of globin caseinate intravenously	No symptoms.
139	Control	4 c.c. of 1 per cent. globin intraperitoneally	Marked symptoms like Nos. 128 and 133.
140	Control	1 c.c. of 0.2 per cent. globin intravenously	Marked symptoms like No. 130; recovered.
141	Control	1 c.c. of 1 per cent. globin intravenously	Severe respiratory symptoms; recovered; chloroformed; hemorrhages in lung and lymph node.

FIXATION EXPERIMENTS.

Rabbits were given repeated injections of globin or globin caseinate and were bled a week or ten days after the last injection. The serum was removed the following day, inactivated at 56° C. for one half hour, and used for fixation experiments. An anticasein serum (No. 101 (rabbit)), which was on hand from previous experiments, and another serum (No. 58), somewhat weaker, were prepared in a similar manner.

IMMUNIZATION PROTOCOLS.

Rabbits Treated with Globin.—Rabbit 55 was given four intraperitoneal injections of a 1 per cent. solution of globin in doses of 20, 10, 10, and 5 c.c., and was bled ten days later. Distinct symptoms of prostration followed each injection, from which, however, the animal recovered entirely.

Rabbit 56 was given one intravenous injection of 1 c.c. of a 1.6 per cent. solution of globin in N/40 hydrochloric acid followed by three intraperitoneal injections of 20, 10, and 5 c.c. Bled ten days later. Symptoms the same as in rabbit 55.

Rabbits Treated with Globin Caseinate.—Rabbit 60 was given four intraperitoneal injections of a 2 per cent. solution of globin caseinate in N/40 potassium hydrate in doses of 6, 10, 10, and 10 c.c. at intervals of four, three, and ten days, and was bled six days later. The animal showed marked symptoms of prostration with polypnea after each injection, but recovered entirely.

Rabbit 66 was given five intravenous injections of a 1 per cent. solution of globin caseinate in doses of 2 c.c. at intervals of four days and was bled nine days later. The animal showed no distinctive symptoms.

With these sera and globin, globin caseinate, and casein as antigens, repeated fixation experiments were carried out. The technique of the experiments is similar in all details to the one we have described previously. It may be noted that two preparations of globin were tested (5) and the globin was tried both in acid and in alkaline solutions (both N/40). The solutions of globin caseinate and casein were prepared in 0.85 per cent. salt solution containing N/40 potassium hydrate. A summary of the fixation results is given in table II. Complete fixation means absence of hemolysis after two hours at 37° C. and standing over night in the ice box.

A study of the results leads to the following conclusions in respect to the comparative antigenic properties of globin, globin caseinate,

and casein: 1. Globin is non-antigenic. 2. Globin caseinate gives rise to an antiserum that reacts with itself and almost equally well with casein. The antiserum also gives a definite fixation reaction with globin. 3. Casein gives rise to an antiserum that reacts with casein and almost equally well with globin caseinate. The chief point of interest is the fact that globin when combined with casein becomes antigenic.

TABLE II.

Fixation Experiments with Globin, Globin Caseinate, and Casein and Various Antisera.

Antigens, 1 per cent. solution, in doses from 0.1 c.c. down.	Minimal fixing dose with various antisera employed in doses of 0.3 c.c.[1]						
	Antiglobin serum 55.	Antiglobin serum 56.	Antiglobin caseinate serum 60.	Antiglobin caseinate serum 66.	Anticasein serum 101.	Anticasein serum 58.	Normal rabbit serum.
Globin I (acid solution)	Negative	Negative	Complete, 0.1 c.c.	Partial, 0.1 c.c.	——	Negative	Negative.
Globin I (alkaline solution)	Negative	Negative	——	Complete,[2] 0.05 c.c.	——	——	Negative.
Globin II (acid solution)	Negative	Negative	Partial,[2] 0.01 c.c.	Partial,[2] 0.02 c.c.	——	——	Negative.
Globin caseinate (alkaline solution)	Negative	Negative	Complete, 0.001 c.c.	Complete, 0.01 c.c.	Complete, 0.001 c.c.	Partial, 0.1 c.c.	Negative.
Casein (alkaline solution)	——	——	Complete, 0.001 c.c.	Complete, 0.02 c.c.	Complete, 0.0001 c.c.	Partial, 0.1 c.c.	Negative.

An investigation as to the possible multiplicity of antibodies in the antiglobin caseinate serum immediately suggested itself. It seemed possible that the antiserum might contain separate antibodies for globin and for casein, and to investigate this possibility absorption experiments were resorted to. The results were only partially conclusive owing to technical difficulties.

[1] The dose of alexin fixed is 1 c.c. of a 10 per cent. solution of fresh guinea pig serum. The hemolytic unit added is 1 c.c. of a 5 per cent. suspension of washed sheep blood sensitized with four minimal hemolytic doses of rabbit anti-sheep serum, usually about 0.001 c.c.

[2] In this instance inhibition to fixation (more or less hemolysis eventually) occurred in higher doses.

Experiment 1.—To 1.5 c.c. of antiglobin caseinate serum 60, diluted with equal parts of sodium chloride solution, was added 0.02 gm. of globin. After remaining at 37° C. for one hour with repeated shaking the mixture was filtered. There was no evidence of solution of the globin. The fixing value of this treated serum was then compared with the same amounts of untreated serum 60, with globin, globin caseinate, and casein as antigens with the results given in table III.

TABLE III.

Fixing Properties of Antiglobin Caseinate Serum 60 before and after Absorption with Globin.

Antigens, 1 per cent. solution, in doses of 0.1 c.c.	Untreated serum.	Treated serum.	Antigens, 1 per cent. solution, in doses of 0.1 c.c.	Untreated serum.	Treated serum.
Globin	Complete	Negative	Casein	Complete	Complete.
Globin caseinate	Complete	Partial			

It is clear from this experiment that the absorption of an antiglobin caseinate serum by globin removes all the fixing property for globin and part of the fixing property for globin caseinate, but none of the fixing value for casein. This seems to indicate the presence in antiglobin caseinate of two antibodies, one of which reacts with globin and one with casein, and both, of course, with globin caseinate. It was unfortunately impossible to prove this conclusively by the reverse absorption experiments with globin caseinate and with casein. This was due to the fact that casein goes almost entirely into solution in serum, and globin caseinate partially into solution, leaving a filtrate which is in itself a fixing combination. Attempts to obviate this solution by adding sufficient N/10 hydrochloric acid to neutralize the serum, with or without subsequent return to normal by the addition of aliquot parts of potassium hydrate after removing the casein, were not successful, as the casein still went into solution in the neutral serum and the antibody for globin, as tested by a control serum neutralized by the acid alone, was apparently destroyed. This latter fact may explain why globin in alkaline solution was better fixed by the antiglobin caseinate serum than when in acid solution.

SUMMARY.

This study of globin and its compound with casein (globin caseinate) shows that globin fails to produce fixation antibodies in rabbits after repeated injections, thus agreeing with our own work

and with that of others with similar histon bodies which are primarily toxic. When globin is combined with casein, however, it gives rise to antibodies that react not only with globin caseinate and casein but also with globin. The antibodies in antiglobin casein serum are apparently separate, one for globin and one for casein. In other words, the change in globin undergone on combination with casein has apparently rendered it antigenic.

We did not succeed in demonstrating the genesis of this new antigenic property by anaphylaxis experiments.

A further investigation of similar and more complex combined proteins is indicated and gives promise of more light on the nature of biological specificity.

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THE RENAL LESION OF EXPERIMENTAL CANTHARIDIN POISONING.*

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PLATE 102.

The interest recently aroused in the study of experimental nephritis by physiological methods, and the tendency to classify the various forms of induced nephritis as either tubular or vascular, has resulted in a thorough investigation of the anatomical changes and physiological reactions in the experimental lesions. With almost unanimous consent the lesions caused by cantharidin and arsenic have been grouped as vascular nephritis, and those caused by potassium chromate, uranium nitrate, and corrosive sublimate as tubular nephritis. The distinction is based chiefly on the presence of striking histological changes in the latter group and their absence in the former, and also on the marked changes, in the first group, in the power of the vessels to respond to sensory stimuli, to adrenalin, and diuretics. Most investigators recognize, however, that while in a given nephritis either vascular or epithelial lesions predominate, the lesions are never exclusively vascular or tubular. The severe or long continued epithelial forms always give eventually the characteristics of the vascular form, and the vascular nephritis always shows some lesion of the epithelial cells of the tubules. This I have expressed elsewhere as follows: "By combining physiological and anatomical methods in the study of nephritis it is possible to distinguish types of nephritis in which either tubular or vascular changes predominate, and are essentially characteristic of the lesion produced, but it is not possible to say that a given poison produces

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exclusively tubular or exclusively vascular lesions."[1] Still, at the time this statement was made I considered cantharidin to be essentially a vascular poison with little power of producing epithelial injury. This is the view also of others (Schlayer and Hedinger, Takayasu, and MacNider) who have attempted to differentiate vascular and epithelial forms of nephritis.

Doubtless all investigators in this field have seen the swollen granular and vacuolated tubular epithelium, with more or less solution or disintegration of the cells, but these lesions have been so slight in contrast with the extensive necrosis of chromate and uranium poisoning that they have been considered as relatively unimportant in view of the very definite vascular disturbance of cantharidin nephritis brought out by physiological methods of study.

A recent chance observation of the occurrence of abundant mitoses in the tubular epithelium in the stage of repair of cantharidin nephritis has, however, caused me to believe that the epithelial lesion is very important. I have therefore examined the material which has accumulated during several years' study of experimental nephritis and have also reviewed the literature of cantharidin nephritis.

It is of interest that Cornil and Brault, in 1884, described mitotic figures in the epithelium of the convoluted tubules in late poisoning (fourth day); but, with the exception of Lyon, in 1904, who also found them (after the fourth day), this has not been noted by later investigators. All investigators describe the presence of red blood cells, lymphocytes or polymorphonuclear leucocytes, or serum, or some combination of these in the capsular space and to some extent in the convoluted tubules (Cornil and Brault, and Eliaschoff). Browicz found a granular material without nuclei in the capsular space and lymphoid cell infiltration in the interstitial tissue. Aufrecht produced in the rabbit a chronic nephritis as the result of daily injections over a period of several weeks.

Degenerative changes in the epithelium of the convoluted tubules are described by all these investigators, and Welch, Lyon, and Opie regard the large pale cells seen in the capsular spaces as desquamated cells arising from the proximal convoluted tubule and not, as did Cornil and Brault and Eliaschoff, as swollen lymphoid cells.

It is in connection with this accumulation of desquamated tubular epithelium that the observation of numerous mitoses is of importance. The combination of desquamation and of repair, as shown by mitoses, indicates severe epithelial injury, and while the degree

[1] Pearce, R. M., Hill, M. C., and Eisenbrey, A. B., *loc. cit.*, p. 223.

of this injury cannot be determined by the study of the desquamated cells, which may pass out with the urine, it can be estimated by the study of the number of mitotic figures in the regenerating tubular epithelium. In a series of kidneys from ten animals (dogs and rabbits), I find four representing lesions later than the third day. Of these, three, one from the rabbit and two from dogs, show numerous mitotic figures in the tubular epithelium. The rabbit represents the third day and the two dogs the fifth day of cantharidin poisoning. The notes on one of these animals follow.

A dog weighing 7 kilos received daily 0.013 gm. of cantharidin in acetic ether, subcutaneously. The urine increased in amount on the second day and then decreased until the fifth day, with increase in specific gravity. Albumin was found in the urine daily. The sediment contained red blood corpuscles, epithelial cells, and hyaline and granular casts. On the fifth day physiological observations were made of the response of the kidney to various stimuli, and on introducing a bladder cannula anuria was found to be present.

Histological examination shows granular degeneration of the cells of the convoluted tubules and of the loops of Henle with free desquamated epithelial cells, more abundant in the loops of Henle than in the convoluted tubules. Such cells are for the most part large and swollen and very granular, with necrotic or pyknotic nuclei, and sometimes with hyaline droplets in the protoplasm. In both convoluted tubules and the loops of Henle mitotic figures are found, but only with difficulty in the latter; in the convoluted tubules they are very abundant. Two and three dividing cells in the cross section of a convoluted tubule are not uncommon and sometimes four or five may be seen (figure 1). The cells undergoing mitosis have a large amount of protoplasm which projects into the lumen and frequently when several such cells are close together the lumen appears to be blocked. No mitotic figures are seen in the collecting tubules. Frozen sections stained by Sudan III and hematoxylin show a moderate number of coarse fat globules in the loops of Henle, and here and there in the convoluted tubules foci of fine fat globules. Casts are only occasionally seen. The glomeruli are congested, but otherwise appear normal.

This extensive repair indicates that the preceding injury to the tubular epithelium must have been very severe, and that however

dominant the evidence of vascular injury may be, as shown by physiological methods, there is a concomitant epithelial destruction of unusual degree. The extent of this epithelial destruction was clearly indicated by Lyon, but his observations have been largely disregarded. He states: "In the rapid poisoning cases there is most diffuse necrosis of the secreting cells. The tubules are dilated, and there is entire disintegration of the inner portion of the cell, so that only the basal portion remains attached to the basement membrane. In this basal portion, which is granular and stains deeply, the nucleus is frequently retained." The incomplete destruction of the cell, that is the persistence of the nucleus, would explain the rapid and profuse mitosis which I have described.

CONCLUSION.

Although the study of experimental nephritis by physiological methods shows that the most striking effect of cantharidin is injury of the blood vessels, the great abundance of mitotic figures in the tubular epithelium in the stage of repair points to an equally widespread and severe epithelial injury. Caution must therefore be observed in ascribing the physiological disturbances of kidney function caused by cantharidin as due exclusively to a vascular injury, and in regarding cantharidin nephritis as a pure type of vascular nephritis.

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EXPLANATION OF PLATE 102.

FIG. 1. The figure shows a convoluted tubule with five mitotic figures and a single mitotic cell of an adjacent tubule. The section is from the kidney of a dog that had received small amounts of cantharidin daily and was chloroformed on the fifth day. 1/12 oil immersion lens; No. 4 eye piece.

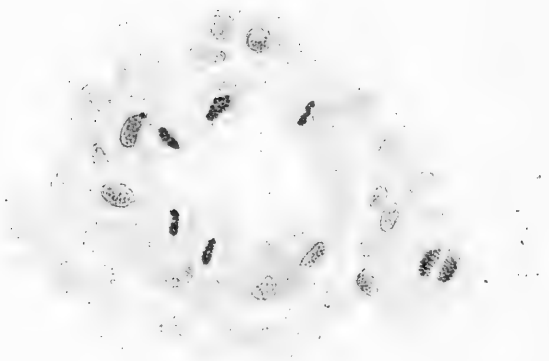

FIG. 1.

(Pearce: Renal Lesion of Experimental Cantharidin Poisoning.)

THE VALUE OF MALLORY'S CONNECTIVE TISSUE STAIN FOR THE DEMONSTRATION OF VARIATION IN THYROID COLLOID.*

By A. P. JONES, M.D.

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The present series of experiments was undertaken to explain certain changes observed by Bunting and Brown[1] in the thyroids of rabbits following bile intoxication. In some of the animals they found the colloid of the thyroid extensively vacuolated and pale staining, and in a few cases they found empty or half empty alveoli in glands which were histologically normal or showed colloid goitre.

The experiments of Bunting and Brown were repeated and similar results were obtained, but no extensive or constant outpouring of colloid was observed, though the colloid frequently showed a pale eosin-staining. It soon became apparent that a more reliable method and one easier to interpret than the varying intensity of eosin staining must be found to indicate what change, if any, had taken place.

With the idea in mind that the outpouring of thyroid secretion following intoxication was the most probable explanation, I sought a staining method which would differentiate the presence or absence of the thyro-iodin in the thyroid colloid. Practically all the more familiar staining methods, and variations of them, were tried, following fixation by alcohol, formalin, saturated bichloride of mercury, and Zenker's fluid. It was found that, following fixation by Zenker's fluid or bichloride, the colloid showed, with variations of conditions, a variable affinity for the different elements of Mallory's connective tissue stain.

* Received for publication, March 10, 1913.

[1] Bunting, C. H., and Brown, W. H., *Jour. Exper. Med.*, 1911, xiv, 445.

A slight modification of the method described by Mallory and Wright[2] was found to give the best results. This modification consists in omitting the acid fuchsin and staining only with the mixture of orange G and anilin-blue. Constant staining conditions were maintained throughout the experiments, and frequently, to insure identical treatment, sections of both control and autopsy portions of the thyroid were placed on the same slide.

The microscopical picture is constant and clear cut, and from the experiments here reported it can be stated definitely that the colloid which contains iodothyrin stains with anilin-blue, and that which does not stains with orange G. Both staining reactions can often be seen in the colloid of the same alveolus.

This staining reaction apparently depends on the fact that orange G has a greater affinity for the colloid than has the anilin-blue, but is decolorized by iodin or the mercuric salts of iodin, while anilin-blue is not affected by iodin and therefore stains the iodin-containing colloid. This can be demonstrated *in vitro*. If a drop of tincture of iodin is added to a dilute solution of orange G, the solution becomes colorless, and this clearing up is greatly hastened by the addition of a drop of saturated bichloride of mercury. A similarly prepared solution of anilin-blue, on the other hand, is unaffected by iodin and bichloride. Further proof that, following this technique, blue staining colloid indicates the presence of iodothyrin, and orange staining colloid its absence, is given by the comparison of the staining reaction of thyroids with their iodin content chemically determined.

It must be borne in mind that the stain indicates only the thyroiodin of the colloid and gives no evidence of that in the thyroid tissue. Also different parts of the gland may vary in structure, and therefore this method is more accurate in smaller thyroids where a conception of the whole may be gained by a few sections. This may account for the apparent exceptions seen in table I, as both human and dog tissue were used. As a means of indicating the staining results, an estimate is made in each case of the total area of blue

[2] Mallory, F. B., and Wright, J. H., Pathological Technique, Philadelphia and London, 1908, 322.

staining and of orange staining colloid, and is represented as blue 2, orange 1; blue 1, orange 10; all blue, etc. The method of iodin determination is that described by Marine and Williams.[3]

TABLE I.[4]

Tissue.	Histological examination.	Staining.	Iodin content per gm. of dried thyroid.
H 689	Colloid goitre	All blue	2.28 mg.
H 690	Colloid hyperplastic	Blue 10 (pale), orange 1	0.34 mg.
H 691	Angiosarcoma	Orange 10, blue 1	0.08 mg.
H 692	Colloid goitre	Blue 2, orange 1 (many empty alveoli)	0.54 mg.
A 233 *b*	Colloid hyperplastic	Blue 1, orange 1	2.77 mg.
A 234 *b*	Early colloid goitre	Blue 1, orange 1	1.62 mg.
C 1	Hyperplastic	All orange	0.15 mg.
C 2	Hyperplastic	Orange 10, blue 1	0.27 mg.
C 3	Hyperplastic	Orange 20, blue 1	0.69 mg.
C 4	Hyperplastic	All orange	0.21 mg.
C 5	Colloid goitre	Orange 10, blue 1	0.66 mg.

In table I specimen *H* 690 appears to present an exception to the rule, but this is explained by the fact that while the blue staining colloid predominates the stain is everywhere extremely pale, so that the low iodin content is not remarkable. Additional evidence as to the reliability of the stain is shown by table II of the iodin-feeding experiments. In these experiments a control portion of thyroid was removed under anesthesia, the rabbits were fed potassium iodide or thyroid extract in capsules for varying lengths of time, and then a second portion was removed, or the animal was killed and the whole thyroid removed. Sections from the portions removed before and after iodin feeding were then stained and compared, with the results given in table II.

It is seen that in every case the area of blue staining colloid is greatly increased after the feeding of iodin. In experiment *T* 34 the control portion showed not only a great predominance of orange

[3] Marine, D., and Williams, W. W., *Arch. Int. Med.*, 1908, i, 349.

[4] For the first six of the specimens I am indebted to Dr. David Marine, of the Lakeside Hospital, Cleveland. The iodin determinations were made by him and sent to Dr. Bunting, who compared them with the estimates obtained by the staining method. For the remaining specimens reported in the table, both staining and iodin determination were done in this laboratory.

staining colloid, but also a histological picture indicating a state of activity, while the portion removed after iodin feeding showed, besides the change of the colloid to blue staining, a histological change to the resting, or colloid goitre stage. This change to the resting stage following iodin feeding has been repeatedly observed by Marine.

TABLE II.

Tissue.	Staining of control.	Iodin feeding.	Staining at autopsy.
T 34	Blue 1, orange 5	20 gr. potassium iodide daily for 4 doses	Blue 8, orange 1.
T 37	Blue 1, orange 15	10 gr. potassium iodide every 10 hrs. for 3 doses	Blue 1, orange 6.
T 41	Blue 1, orange 20	10 gr. potassium iodide daily for 4 doses	Blue 1, orange 10.
T 42	Blue 1, orange 5	10 gr. potassium iodide daily for 4 doses	Blue 3, orange 1.
JT 1	Blue 1, orange 5	1.8 gm. thyroid extract in 5 days	All blue.
JT 2	Blue 1, orange 6	1.8 gm. thyroid extract in 5 days	All blue.

Soon after beginning to use this stain, it was observed that any shock to the rabbit, such as prolonged anesthetization, severe operation, or convulsions, caused a diminution of the blue staining colloid and a corresponding increase in the orange staining area. This was interpreted to mean an outpouring of thyroid secretion. Therefore, by arguing backwards from the symptoms of exophthalmic goitre where hyperactivity of the thyroid is followed or accompanied by tachycardia and muscular tremor, an attempt was made to determine the effect of tachycardia and excessive muscular action on the thyroid.

TABLE III.

Section of Both Vagi.

No. of experiment.	Staining of control.	Staining at autopsy.
T 30	All blue	All orange.
T 31	All blue	Blue 5, orange 1; many alveoli empty.
T 36	All blue	Orange 20, blue 1.
T 39	All blue	Orange 4, blue 1.
T 40	Blue 5, orange 1	All orange.

For the production of tachycardia, rabbits were anesthetized, a control portion of thyroid was removed, and both vagi were cut as low down in the neck as possible, so as to avoid the nerve supply of the gland. The animals lived from twenty-four to forty-eight hours after operation, dying finally from lung involvement. The results were constant and striking.

The experiments with prolonged or excessive muscular action have not yet been completed and will be reported later.

From the above experiments the conclusion seems warranted that greatly increased activity of the heart is accompanied by an outpouring of iodothyrin from the thyroid colloid. The experiments with excessive muscular activity which have been so far carried out give similar evidence. In this connection may be mentioned the case of a dog that had been previously used for experimental purposes, the experiment being carried on for a period of four hours with the animal under ether anesthesia. The thyroid of this dog showed large vesicles filled with colloid which was almost entirely orange staining, and the chemical analysis showed an extremely low iodin content.

It is not within the province of this paper to attempt to discuss the function of the thyroid, but the experiments cited seem to show a relation between the outpouring of iodothyrin from the thyroid colloid, and the increased muscular action. The experiments seem to indicate further that the colloid of the follicle serves as a matrix or menstruum for the storing of the iodin-containing principle of the gland in much the same way as the hemoglobin is held by the stroma of the erythrocyte. However, the complete outpouring of the colloid of the alveoli, often observed in experimental work on the rabbit, but due to factors not yet ascertained, might indicate that in the colloid itself are further important secretions of the thyroid cells, or else that it is the means of rendering available to the organism the last vestige of iodothyrin held in the acini.

In conclusion I wish to express my thanks for the criticisms of Dr. C. H. Bunting, at whose suggestion the experiments were undertaken.

CONCLUSIONS.

1. Mallory's connective tissue stain used in connection with fixation of tissue by Zenker's fluid or bichloride of mercury demonstrates the presence or absence of iodin in the thyroid colloid, as is evidenced by parallel chemical determinations, iodin feeding, and test-tube experiments.

2. Greatly increased activity of the heart is accompanied by a loss of thyro-iodin from the colloid of the thyroid.

3. This method, while apparently not adaptable to clinical use, may prove of value in further experimental work on the thyroid.

THE RESULTS OF THE SERUM TREATMENT IN THIRTEEN HUNDRED CASES OF EPIDEMIC MENINGITIS.*

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I.

The serum treatment of epidemic meningitis, developed during the prevalence of the pandemic beginning in 1904 and not yet wholly at an end, has now been subjected to a rigorous test extending over a period of six years and embracing many different countries. The results of this trial have thus far been published in part only. The present publication deals with the analysis of the histories of cases, about 1,300 in number, that were treated with the antimeningitis serum prepared and distributed by the Rockefeller Institute. The histories have been collected not only from many different parts of the United States and Canada but also from several European and Asiatic countries to which the serum was sent. The large number of the cases and the diversity of the circumstances under which the serum came to be administered give to the following presentation a significance greater perhaps than that afforded by a strictly local report.[1]

BIOLOGICAL DISTINCTIONS AMONG MENINGOCOCCI.

The bacteriological cause of epidemic meningitis is admittedly *Diplococcus intracellularis*. Formerly no fundamental biological distinction was made between meningococci; now differences of

* Received for publication, March 1, 1913.

[1] The 1,300 cases include the 712 cases previously analyzed (*Jour. Am. Med. Assn.*, 1909, liii, 1443). The number of physicians and hospitals making the reports is too great to enable me to thank them individually. In a previous paper (*Jour. Exper. Med.*, 1908, x, 690) many acknowledgments were made, to which I desire now to add the name of Professor A. Netter, of Paris, for his invaluable services.

virulence for animals, degree of digestibility in leucocytes, and power of resistance to solution by immune serum are being recognized. The pathogenicity of the meningococcus for laboratory animals is not great, but by the intraperitoneal injection of young guinea pigs the virulence can be tested and different strains more or less pathogenic can be distinguished. Some strains maintain a given virulence for a year or more and others lose this quality very soon after being obtained from human sources. Rarely a strain that shows no virulence for guinea pigs takes on suddenly a heightened activity. There is no sharp agreement in pathogenicity between strains that are virulent for guinea pigs and those virulent for monkeys or man. Some strains, but not all, can be adapted by successive inoculation to guinea pigs and to monkeys.

Opsonic experiments have disclosed the fact that meningococci derived from different sources are digested by leucocytes with varying facility. The kind of leucocytes employed appears unimportant and even the sera, whether normal or immune, play no obvious distinguishing part; the variations depend upon the strain of meningococcus. All meningococci are readily phagocyted in the presence of serum and dissolved ultimately by leucocytes; but while many strains are quickly digested, a small number resist disintegration far more tenaciously. In estimating the content of opsonizing substances in different samples of antimeningococcic sera the strains digesting slowly should be chosen.

Whether in meningitis in man a similar variation among meningococci occurs naturally can only be inferred. Exudates, as obtained from different cases of epidemic meningitis, differ in number and distribution of the contained meningococci. At one time the number of meningococci is small and at others large, and sometimes the meningococci lie chiefly within cells, while at others they occur mainly free in the fluid.

Under the influence of the antimeningitis serum injected into the subdural space the diplococci come, as a rule, to lie more and more within the leucocytes. As recovery from meningitis progresses, even where no antiserum has been employed, a corresponding phenomenon is noted. Moreover, the meningococci phagocyted under the influence of the serum undergo an accelerated disintegra-

tion. In how far resistance to solution in leucocytes is due to the quality of fastness of the meningococcus to the action of the intraleucocytic enzymes is not certainly known; nor is it known definitely that the less readily englobed meningococci consist also of strains that are more or less resistant to immune opsonins or bacteriotropins. But indications exist supporting the view that certain examples of epidemic meningitis in man which respond imperfectly to the therapeutic action of the serum are caused by strains of meningococci fast to the antiserum employed. These strains tend to remain persistently extracellular and to multiply freely in the presence of the antiserum, even under conditions of its high concentration in the subdural space. The cases of human infection with the supposedly resistant strains fail not only to respond to the therapeutic action of the antiserum but can be inferred also not to ameliorate spontaneously and thus tend to a fatal termination. Certain biological differences have been noted among meningococci from different sources. These relate to the readiness with which they can be cultivated, to survival in cultures, and fluctuation in power to ferment carbohydrates. The variations in opsonic and leucocytic reactions would seem to be of a more fundamental character, just as they are of greater significance in respect to the pathogenic power of the meningococcus and to better control of epidemic meningitis by the employment of the antimeningitis serum.

MORTALITY OF EPIDEMIC MENINGITIS.

The establishment of the bacterial cause and the perfection of lumbar puncture made it possible, as never before in a widely spread epidemic of meningitis, to ascertain accurately the degree of its fatality. Mortality estimates based on earlier epidemics are far less trustworthy since they obviously could not have rested on such certain evidence of specific disease. The pandemic of 1904–9 afforded, therefore, the first opportunity to determine the actual mortality of epidemic meningitis as it affects countries and peoples of various nationalities and widely different social customs. Accurate knowledge of the mortality becomes imperative once we undertake to determine the value of the serum treatment. Com-

prehensive and accurate figures are available from the United States and Canada, Great Britain and Ireland, Germany, France, Belgium, and Italy, and Asia and Africa. The facts to be given immediately relate exclusively to the disease as it prevailed either before the serum treatment was introduced, or where it had not yet been applied.

In the period from 1904 to 1909 epidemic meningitis prevailed over a wide territory in the United States and Canada, and after an intermission reappeared in the winters of 1911–12 and 1912–13 in the Southwestern States. The fatalities recorded at several places are as follows: Greater New York City, 73 per cent.; Boston, 69 per cent.; Hartford, 76 per cent.; several cities in Ohio, 70 to 90 per cent.; Porterville, California, 90 per cent. During 1911–12 more than 1,500 cases arose in Texas and Louisiana among which (exclusive of cases treated with serum) the mortality was 75 per cent. or more.

The epidemic in Great Britain was equally severe. The mortality reports include Leith, 74.7 per cent.; Edinburgh, 80 per cent.; Glasgow, 74.8 per cent., and Belfast, 70 per cent.

The largest German figures relate to the Silesian epidemic. The lowest mortality reported is based on a group of 47 cases at Brieg and is 42.5 per cent., while in Breslau it was 62.3 per cent., and among the 3,085 cases collected by Flatten, 67 per cent.

The mortality in France was placed by Netter at 75 per cent.; in Belgium it is given at 77.7 per cent.; while the degree of fatality in Italy may be deduced from the figures for Milan which are 55.6 per cent.

The reports from Asia and Africa are few but they indicate severe infection. In Palestine the mortality was above 80 per cent.; in Greece (Athens) 58.6 per cent.; and in the Transvaal 74 per cent. Although accurate figures are not available, the epidemic prevailed in Java and in German East Africa.

The statement of von Ziemssen may be taken as a criterion of the views held in prebacteriological days of the severity of epidemics of meningitis. He states that the mortality fluctuates between 30 and 70 per cent., the average being about 40 per cent. There is, of course, something highly artificial in arriving at an average in

this manner, and the artificiality is brought out strikingly by the reliable figures of the recent pandemic.

There appears to have been little variation in the mortality according to the period at which the epidemic flourished, and as it affected the inhabitants of the mining districts of Northern Germany or the Transvaal, the industrial populations of Ireland or Scotland, the general population of France, the military garrisons of Belgium, the urban population of New York or Boston, the suburban population of remote districts in Ohio, California, Texas, and Louisiana, or the agricultural and artisan peoples of Greece and Palestine. Everywhere the upper limits of von Ziemssen's figures were reached or surpassed. Hence it appears that the virulence of the meningococcus was the chief factor in causing death, and differences among peoples, as represented by race and occupation, were far less conspicuous factors.

Meningococcus meningitis prevails also as a sporadic affection. The mortality of the sporadic disease is far more difficult to determine than that of the epidemic disease for the reason that vital statistics covering wide areas are difficult to obtain. Such data as are available tend to show that the sporadic disease, when bacteriologically diagnosed, is less fatal than the epidemic disease. Posterior basic meningitis, which is probably merely a variety of sporadic meningitis, has a less general distribution. This disease had claimed attention chiefly in England. The fatality of these affections may fall as low as 40 to 50 per cent.

MODE OF TERMINATION.

Cases of meningitis are very varied both in respect to their intensity and evolution. In rare individual instances the disease sets in severely and yet terminates in recovery, sometimes in a few days, or the onset is mild although the infection later develops an alarming gravity; while again the disease progresses slowly or continuously through weary weeks or months to end usually, but not always, in death. The cases of ordinary severity pursue a middle course, while the fulminant ones arise and terminate within a few to forty-eight hours. There remains still another variety of the disease hitherto little remarked and the frequency of which is not

known; namely, the ambulant. The subjects are commonly little ill at any time, so that recovery is the rule. Nevertheless, in rare instances death may suddenly supervene, and somewhat more often the slight illness increases in severity until the ordinary type is evolved.

Neither the symptoms nor the mode of their evolution support a prediction as to the probable outcome of the disease. Several different types have been constructed out of the variety, complexity, and degree of severity of the symptoms, but a sharp discrimination between them is often wanting in practice. For this reason observation of individual cases affords little final information concerning the nature of epidemic meningitis, and the outcome of single cases is an uncertain guide to the value of a given therapeutic measure.

Death may be considered as taking place under three sets of circumstances: (*a*) rapidly, after sudden onset of illness, as in meningitis siderans, or so called fulminating meningitis; (*b*) through a gradual intensification of the symptoms and after a duration of from six to ten days; (*c*) slowly, in the course of which the acute stage has been survived and after the condition has become chronic.

Recovery, also, when it occurs is not by a single mode of termination, but by at least two modes. The transition to convalescence and recovery is generally by a process of gradual amelioration of the symptoms, or lysis. Sometimes, but more rarely, the transition takes place abruptly by a sudden or critical cessation of the symptoms that follow quickly upon the onset of the infection or after several days have elapsed.

Knowledge is lacking of the relative frequency with which the several types of meningitis occur in different epidemics. What would be particularly informing is accurate knowledge of the frequency of the extreme cases—the fulminant and the abortive, latent, rudimentary, or ambulant ones, so called. The throwing together of the abortive and ambulant cases is not strictly justified by their usually mild character, since the one sets in sharply and the other begins insidiously. While the former tends to terminate in recovery by crisis, the latter attracts attention by suddenly pass-

ing into the severe or even fulminant disease. That such mild cases of epidemic meningitis occur has now been conclusively shown by bacteriological examination of fluids removed by lumbar puncture. Exceptionally an epidemic has been composed mainly of them, as in Kehl. In many epidemics they have wholly escaped detection. Apparently their occurrence is infrequent, and excellent observers of large numbers of cases of epidemic meningitis have not encountered them.

The proportion of fulminant to ordinary cases can be inferred rather than asserted. At the beginning of a severe epidemic they appear to be more frequent than at the end. Certain epidemics have been noted for their absence. On the other hand, fulminant cases have appeared in associated groups, one case following another in rapid succession in barrack, school, or family. Some idea of frequency can be inferred from the duration of the illness preceding death. The chief danger in this inference arises from the uncertainty surrounding the period of onset of some of the cases. Among 1,481 fatal cases analyzed by Flatten, 29 died within six, 30 in from six to twelve, and 53 in from twelve to twenty-four hours of the appearance of symptoms. That is, 112, or 9 per cent., succumbed within the first twenty-four hours, while another 111 cases terminated fatally in the second twenty-four hours, giving 18 per cent. of the whole as having suffered from the fulminant disease.

DURATION OF THE DISEASE.

The duration may be considered according to the period elapsing before either death or recovery occurs. Among the 1,481 fatal cases just mentioned, 361, or 25 per cent., terminated in the first three days; 351, or 24 per cent., from the fourth to the seventh day; and almost 50 per cent. later than the seventh day of illness. Hence, it cannot be maintained that the survival of the first week of the infection affords great insurance of ultimate recovery. One half of the cases surviving the first week terminated between the fifth and eighth weeks, while 7 per cent. (107 cases) survived eight weeks or longer. Among Schneider's 80 fatal cases, 26, or 32.5 per cent., succumbed in the first three days; 18, or 22.5 per cent., in the next four days; and 36, or 45 per cent., later than the first week.

Viewed from the side of recovery the period of duration of the disease has fluctuated with the type. The abortive cases are said to terminate in recovery in from one and one half to five days (von Ziemssen, Strümpell). Strümpell states that the critical period falls between the fifth and tenth days, when either death results or convalescence begins. Schneider's analysis of 52 cases shows 3 recoveries from the fifth to the ninth day, 26 from the eleventh to the fortieth day, and 45 from the forty-first to the one hundred and tenth day. Rieger discharged 25 patients from the hospital on the average of the fortieth day. Heubner states that the average duration of the disease terminating in lysis is three to four to six weeks; von Ziemssen gives for favorable cases two to three weeks, and for less favorable ones three to four weeks. Holt states that in the New York epidemic of 1904–5, among 350 cases which recovered, the duration in 3 per cent. was one week or less, and in 50 per cent. five weeks or longer.

Thus we may conclude that in the recent past the tendency of epidemic meningitis has been to terminate in death, and of the fatalities about one half arose in the first week of the illness. On the other hand, among the recoveries a small percentage took place in the first week, and almost all after several weeks or months of illness.

INCIDENCE AND MORTALITY ACCORDING TO AGE.

Young persons are more subject than older ones to epidemic meningitis. This conclusion, arrived at by Hirsch and others, is supported by recent more accurate studies. Certain epidemics affect children almost entirely, and nurslings rather than older children (Hirsch, Heubner). Among the 2,916 cases in Silesia in 1904–5 analyzed by Flatten, 247 arose in the first, 239 in the second, 293 in the third, 334 in the fourth, and 271 in the fifth year. Hence about one half (1,384) of the cases occurred in the first five year period, one third (844) in the second, one eighth (338) in the third corresponding period, and the remainder (350) later than the fifteenth year of life. Göppert noted among 629 cases 102, or 17 per cent., in nurslings. Since in children below three years of age the symptoms of meningitis are often masked, it follows that the

bacteriological diagnosis accounts for the greater number of infants shown to be attacked during the pandemic.

The mortality is affected by the age of the attacked. Hirsch states that the disease is most fatal in children and in adults after the fortieth year. During the epidemic in New York the mortality among infants of two years and under approximated 100 per cent. Flatten's figures based on the Silesian epidemic show approximately the same result. The number of cases analyzed was 2,976. They may be divided as follows: under one year, 255 with 89 per cent. of deaths; under two years, 243 with 83 per cent. of deaths; between two and five years, 866 with 72 per cent. of deaths; between five and ten years, 920 with 62 per cent. of deaths; between ten and fifteen years, 346 with 56 per cent. of deaths; between fifteen and twenty years, 187 with 63 per cent. of deaths; between twenty and thirty years, 76 with 59 per cent. of deaths; and between thirty and seventy years, 83 with 83 per cent. of deaths.

Hence, in the past, epidemic meningitis has been most fatal to infants and adults after the third or fourth decennium, while the intermediate age periods have been somewhat more favorable to spontaneous recovery. The favorable period begins to be apparent about the fifth year and endures until the twentieth or thirtieth year, when it diminishes with increasing rapidity as age advances.

COMPLICATIONS AND SEQUELS.

We shall consider briefly the chief complications and sequels only. The common secondary infections of the respiratory tract, etc., will be neglected. In some instances the meningococcus causes a complicating pneumonia, endocarditis, or serositis exclusive of the joints; in very rare instances it has produced general infection or septicemia.

Of greater importance are the involvements of the central nervous system, peripheral nerves, and sense organs in the infective process. True motor paralyses occur infrequently and permanent ones are rare. Of the nerves of the head the facial and hypoglossal are oftenest affected. The frequency has varied with different epidemics and the complication has been noted in abortive as well as severe cases and tends to arise at the end of the first week. Hemi-

paresis, a rare condition, affecting an arm and less often a leg, comes on usually later than the second week. These paralyses, regarding which figures are scant, are attributed to focal lesions in the brain and spinal cord extending inward from the meninges.

The ear complications are numerous. Both the middle and internal ear may be affected. Inflammation of the middle ear is frequent and increases with the duration of the illness. Thus 35 per cent. of the inflammations arise in the first, 70 per cent. in the second, and 59 per cent. in the fourth to the sixth week (Göppert). Recovery tends to be complete. Inflammation of the internal ear is far more serious. The frequency varies in different epidemics. Percentages range from 33 (Mannkopff and Heller), 16 (Göppert), and 12 (Bauer). Probably 12 per cent. approximates the average among large numbers of cases (Göppert). The involvement usually occurs early in the attack and arises even in the abortive disease. Both ears tend to be affected and hearing is often already lost when the patients enter the hospital. Among sixty-four cases Moos observed affections of one side in three. When the accident occurs in infancy deaf-mutism results. Following epidemics of meningitis the number of deaf-mutes in institutions has greatly increased.

The eyes may suffer slightly or severely, and injury may be temporary or permanent. Muscular affections are common (12 per cent.), ptosis is rare, and pupillary irregularities are frequent (24 per cent.); all tend to be temporary, except ptosis. Iritis and iridochoroiditis, retinitis, and periretinal hemorrhages all arise but often disappear without causing permanent injury. Keratitis, with or without ulceration of the cornea, occurs, followed either by recovery or loss of the eye. A condition of basilar amaurosis, from which slow recovery has taken place, has also been described (Göppert). The most serious complication is metastatic ophthalmia, due to the meningococcus, affecting one eye or both, and leading to destruction of the ball. Eye complications have been a variable factor in different epidemics. Göppert reports 14 instances of metastatic ophthalmia (affecting both eyes in 4, and one eye in 10 patients) among 136 cases (10 per cent.). Uhthoff states that among 110 Silesian cases lesions of the optic nerve occurred in 17, metastatic

ophthalmia in 4, and keratitis in 3. Morax noted iridocyclitis in from 3 to 6 per cent. of cases during the Parisian epidemic.

The higher intellectual centers of memory, intelligence, and speech suffer also, but while slight degrees of affection from which recovery takes place are common, only rarely does permanent incapacitation result. Actual mental defects of permanent character arise, according to Göppert, in from 1 to 3 per cent. of the recovered. Looft reports that among 539 Norwegian idiots, 3.7 per cent. were caused by epidemic meningitis.

Finally, hydrocephalus arises very often, especially among the young. In Silesia its occurrence was noted in one eighth of all cases. Recovery may occur without mental defect even where the degree is considerable. Deafness and hydrocephalus are frequently associated, but not as cause and effect.

II.

We may now proceed to consider what changes have been effected in the course and consequences of epidemic meningitis by the employment of the antimeningitis serum. The serum began to be employed in 1906–7, first locally and later more generally. Thus it failed to be used in certain places because the epidemic wave had passed, and in other places its employment was begun at the middle period or height of the epidemic, according to circumstances. There occurred coincidentally, almost without exception, cases of equal severity in which the serum was not administered. These served as checks, or controls. The mortality in them did not vary essentially from the figures already given for different countries page 556). The analysis to follow is based upon cases treated in America, Europe, Asia, and Australia with the serum which was prepared at the Rockefeller Institute by a uniform method and widely distributed, and they therefore present at least this common factor. The chief variable was the experience or lack of experience in those charged with the administration of the serum, which is sometimes reflected in the results achieved. But since this factor will always operate in some degree, the results probably approach the normal.

The high degree of variability of the clinical course of epidemic

meningitis has been pointed out; hence it will not be as profitable to study the influence on individual cases as to consider the effects on the mortality and some other objective features of the disease.

MORTALITY OF SERUM-TREATED CASES.

The number of cases to be subjected to analysis is 1,294. They have not been selected and a single exclusion has been practised. In order to deny no one the benefits of the treatment where available, a small number of hopeless and dying persons were injected. When they survived the injection merely a few hours—less than twenty-four at the maximum—they were not included. The fulminant cases have been included in the analysis and, as will appear, they now seem not to be without the sphere of influence of the serum. The general mortality is shown in table I.

TABLE I.

Mortality of Serum-Treated Cases.

No. of cases.	Recovered.	Died.	Per cent. died.
1,294	894	400	30.9

That the period of injection in the course of the disease affects the fatality has been previously shown.[2] The larger number of cases has been analyzed from this point of view and in accordance with the arbitrary periods previously employed. The results are shown in table II.

TABLE II.

Mortality according to the Period of Injection of the Serum.

Period of injection.	No. of cases.	Recovered.	Died.	Per cent. recovered.	Per cent. died.
1st to 3d day	199	163	36	81.9	18.1
4th to 7th day	346	252	94	72.8	27.2
Later than 7th day	666	423	243	63.5	36.5
Totals	1,211	838	373	69.2	30.8

The pronounced influence of the serum is strikingly shown by these tabulations which serve to confirm the earlier figures given.[3]

[2] Flexner, S., *Jour. Exper. Med.*, 1908, x, 699.

[3] Flexner, S., *ibid.*, pp. 700–1.

In some instances the precise period of first injection of the serum was not stated in the histories, which explains the smaller number of cases analyzed in table II. This table not only establishes the fact that the earlier the serum injections are begun the better the results, but it emphasizes the too common delay in making the diagnosis of epidemic meningitis or of bringing the majority of cases under the specific treatment. Less than one sixth of the cases had the benefit of early injection, and one half received the first injection later than the first week of illness. Doubtless as the serum is made more generally available and as confidence in its value extends, these disadvantages will be more and more removed.

That the mortality is affected by the age of the patient is commonly admitted. The influence of the serum on patients of different ages is shown in table III.

TABLE III.

Mortality according to Age.

Age.	No. of cases.	Recovered.	Died.	Per cent. recovered.	Per cent. died.
Under 1 yr.....	129	65	64	50.4	49.6
1 to 2 yrs.....	87	60	27	69.0	31.0
2 to 5 yrs.....	194	139	55	71.6	28.4
5 to 10 yrs.....	218	185	33	84.9	15.1
10 to 20 yrs.	360	254	106	70.6	29.4
Over 20 yrs.	288	180	108	62.5	37.5
Age not given...	18	11	7	61.1	38.9
Totals.....	1,294	894	400	69.1	30.9

TABLE IV.

Mortality according to Age and Period of Injection.

Age.	Injected 1st to 3d day.				Injected 4th to 7th day.				Injected later than 7th day.			
	No. of cases.	Recovered.	Died.	Per cent. died.	No. of cases.	Recovered.	Died.	Per cent. died.	No. of cases.	Recovered.	Died.	Per cent. died.
Under 2 yrs,.....	13	12	1	7.7	37	28	9	24.3	159	81	78	49.1
2 to 5 yrs.....	30	24	6	20.0	66	49	17	25.8	93	63	30	37.3
5 to 10 yrs.....	55	49	6	10.9	69	61	8	11.6	77	61	16	20.8
10 to 20 yrs.....	67	58	9	13.4	106	73	33	31.1	171	115	56	32.7
Over 20 yrs.....	34	20	14	41.2	65	39	26	40.0	164	101	63	38.4
Totals.....	199	163	36	18.1	343	250	93	27.1	664	421	243	36.6

Table IV gives an analysis of the data of the preceding tabulation according to the period of the disease at which the serum injections were begun.

The age factor affects the outcome of the serum treatment just as it affects spontaneous recovery, only its influence is even more marked in the former. The most favorable cases for the specific treatment fall between the five and ten year period and the least favorable ones fall in the period above twenty years. The favorable period doubtless extends to and even beyond the twentieth year although the analysis given fails to make an exact discrimination for these ages. To subdivide farther the periods risks increasing the statistical error inherent in small numbers; but the reports of certain hospitals covering cases arising during the third decennium indicate no great decrease in response to the serum. What is particularly impressive is the change wrought in the fatality among infants, and while the astonishing result for the first injection period can scarcely be maintained through the vicissitudes of large numbers of cases, yet the outlook is highly promising. The exact coincidence of the favorable ages for spontaneous recovery and for recovery under the serum treatment indicates that the serum provides artificially the means that the body itself employs when it can fabricate them to suppress the infection, and that two factors, one provided by the body and the other by the serum, coöperate in the end result. The change wrought in the manner of termination of the disease enforces the same conclusion.

MANNER OF TERMINATION AND DURATION.

The usual mode of spontaneous termination of epidemic meningitis is by gradual subsidence of the symptoms, or lysis, and the exceptional mode is by abrupt cessation, or crisis. While precise figures showing the number of cases subsiding by crisis in ordinary epidemics are not available, yet it can be said that it has never been observed to be large and that sometimes this class of cases has been wholly absent. The histories of 830 cases embraced in our tabulation permitted of a judgment as to the manner of termination, and it was ascertained that in 30 per cent. the cessation was abrupt, or by

crisis. In our previous tabulation of 400 cases[4] the percentage was placed at 25, which apparently was too low.

A consideration of the manner of recovery according to the age groups brings out some facts of suggestive interest. The data are given in table V, from which it will be seen that among cases of all ages termination by crisis and by lysis occurs about equally when the injection of the serum was begun in the first three days of illness, while when the injection was begun later the number of cases terminating by lysis rose steadily. One other point is indicated by the tabulation; namely, that the proportion of cases that resolve by crisis is highest in the age periods more favorable, and lowest in those less favorable to recovery, whether spontaneously or through specific treatment.

TABLE V.

Recovery by Lysis or Crisis according to Age Periods.

	1st to 3d day.	4th to 7th day.	Later than 7th day.	Total.
Under 1 yr.				
By lysis	3	6	36	45
By crisis	2	6	8	16
1 to 2 yrs.				
By lysis	4	10	26	40
By crisis	3	6	8	17
2 to 5 yrs.				
By lysis	10	31	53	94
By crisis	14	18	10	42
5 to 10 yrs.				
By lysis	26	43	43	112
By crisis	23	18	19	60
10 to 20 yrs.				
By lysis	28	46	92	166
By crisis	30	27	23	80
Over 20 yrs.				
By lysis	11	22	85	118
By crisis	9	17	14	40

With the manner of termination is obviously bound up the period of duration of the active symptoms of the disease. Epidemic meningitis pursuing its usual or typical course passes through a series of reinfections of the cerebrospinal meninges, whence its intermittent or remittent character. To determine, therefore, just

[4] Flexner, S., and Jobling, J. W., *Jour. Exper. Med., loc. cit.*, p. 690.

what constitutes a relapse of the infection is sometimes difficult or even impossible. The most trustworthy guide, next to long and complete intermission of symptoms, is afforded by bacteriological examination of the cerebrospinal liquid, since the meningococci tend not to disappear wholly during the intermissions, while they do so disappear between true relapses. Having in mind these criteria we have separated from the 1,294 cases 56 that showed relapses after greater or less intervals of time. Prompt resumption of the serum injections often but not invariably sufficed to control and suppress the reinfections. Of the 56 cases 40 recovered and 16 died. Among them the mortality was 28.6 per cent., indicating that the relapses respond somewhat less well to the serum than the primary infections.

RESPONSE OF RESISTANT OR FAST STRAINS.

In some instances the meningococci having been at first readily controlled by the injections appear to develop resistance or fastness to the serum. This has been noted in certain cases of relapse going on to a fatal termination. Hence under special circumstances the meningococci seem to acquire a serum-fastness that thwarts its specific action. A variety of serum-fast meningococci exists in a small number, at least, of cases of epidemic meningitis before the serum has been applied. This original fast strain seems not to be of common occurrence and its biological properties have not been minutely studied. It is not known whether serum fastness and indisposition to opsonization and leucocytic digestion go hand in hand or not. Nor is it known whether merely one or many fast strains distinct from one another exist in nature. Information on these points is highly desirable, as is the utilization of fast strains of the meningococcus in the preparation of the antimeningitis serum. Should a true polyvalent serum of high titre carrying immune bodies for the fast strains be developed it is probable that the mortality percentages would come to be depressed still further.

COMPLICATIONS AND SEQUELS.

The number of cases here analyzed should serve to indicate the frequency with which the usual complications and sequels may be met with under the serum mode of treatment. Our earlier tabula-

tion suggested that the recoveries would, as a rule, be complete and the one severe sequel that would still remain, although in diminished degree, is deafness. It is impossible to know whether the histories returned noted all the sequels, but it is probable that the severer ones, which we are considering, were uniformly recorded. I have tabulated the reported instances of impaired hearing and vision and arthritis in relation to the day of the disease on which the first injection of serum was made. The circumstance that sometimes the defect already existed when the injection was made can be disregarded, since, as this fact cannot always be established and as a specific form of treatment may be presumed to be capable, at times, of suppressing the secondary localizations of the meningococci, no exclusion should be practiced.

TABLE VI.

Impaired Hearing and Vision and Arthritis in Serum-Treated Cases.

Day of disease of first serum injection.	1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	2d week.	3d-8th week.	Totals
No. of cases in which deafness occurred	1	15	6	5	3	3	1	7	4	45
No. of cases in which impaired vision occurred	1			2	2			3	4	12
No. of cases in which arthritis occurred		5	3	1				2		11

The instances of arthritis all recovered, as is the rule; and the recovery could be hastened by injecting the serum directly into the inflamed joint. Deafness was complete in 39 and partial in 6 of the 45 instances. Blindness occurred 3 times, some degree of impairment of vision 6 times, iridocyclitis, choroiditis, and metastatic ophthalmia (affecting one eye) once each. Impaired mentality occurred 3 times; in one instance the state is described as imbecile. Muscular paralyses were recorded in 11 histories as follows: strabismus in 5, paralysis of the lower extremity in 3, of the face in 2, and of the shoulder in 1. Deafness and impaired vision were combined in 2 cases, imbecility and impaired vision and arthritis and choroiditis occurred in 1 case each.

The severe and permanent sequels are those resulting from affections of the internal ear and the essential structures of vision. The former arose in about 3.5 per cent. of the serum-treated cases.

This constitutes a marked reduction of the percentages that have been observed among the spontaneously recovered; whether they can be still further diminished by a more prompt and vigorous employment of the serum cannot be predicted.

DISCUSSION.

The foregoing analyses demand merely a brief discussion since they carry their own forceful argument. They show indubitably that the mortality of epidemic meningitis can be greatly reduced by the application of the specific serum treatment and the extent of the reduction is determined by two main factors: the period of the disease at which the subdural injections of the serum are begun and the ages of the persons affected. In view of the fact that the average mortality during the pandemic was approximately 70 per cent., the gross reduction was somewhat less than two thirds. On the other hand, the statistics presented indicate that general early diagnosis and prompt institution of the serum treatment are capable of still further depressing the mortality.

Although at first regarded as doubtful it now appears that fulminant cases of epidemic meningitis are not wholly without the sphere of beneficial influence of the serum. This conclusion rests, first, upon specific statements of recovery of such cases under the influence of the serum, and, next, upon the definite change wrought by it in the mortality of the disease during the first three days of prevalence which is the period during which the fulminant cases terminate in death. Flatten's figures (page 559) and the figures given in table II afford a basis for comparison covering this point.

Undoubtedly it is the ordinary type of epidemic meningitis that responds best to the specific treatment. This fact is shown not only by the general reduction in mortality, but also by the common alteration of the manner of its termination from lysis to crisis. It is just this transformation and the general shortening of the whole course of the disease and the consequent suppression of the chronic form of the infection that bring conviction of the value of the serum not only by reason of statistical presentations but even more cogently to the hospital physicians who have large experience with the usual course and tendencies of the malady.

Other objective criteria of the action of the serum exist. One of the most conclusive is the effect exerted upon the meningococci through which multiplication is arrested and phagocytosis and intraleucocytic digestion accelerated. This impressive evidence of the action of the serum has been recorded many times by different physicians in the histories upon which this paper is based

The complications and sequels are reduced in number. Indeed, the number of permanently injured among the serum-treated has become very small. Of all the severe sequels deafness has remained least influenced. Unhappily, injury to the internal ear takes place very early and sometimes before the diagnosis of meningitis has been made. In view of the great reduction in other severe effects we are permitted to hope that even this one may be diminished by a more uniformly early application of the serum. The arthropathies have not only been reduced in frequency but they have been shown to be amenable to direct injections of the serum. Undoubtedly the tendency to hydrocephalus in the young has been diminished, and the intraventricular injection of the serum has operated in several instances to abolish infection and inflammation of the cerebral ventricles and to reëstablish communication between the ventricles and the subdural space of the spinal cord. Recovery, therefore, has been rendered complete.

III.

ADDENDUM.

In the preceding presentment the value of the serum treatment is based wholly upon the experience gained by the Rockefeller Institute during the several years it was engaged in preparing and distributing the antimeningitis serum. Three reports on the subject have been issued at different times; this report is the final one, since the Institute no longer prepares the serum and is collecting no more histories of cases treated with it. During the period of several years in which the serum was on trial, reports from other countries have also been published. The most important possibly are those of Levy and Netter. They support the value of the serum treatment, as do the many briefer ones published. Within the past

two years two widely remote epidemics of meningitis came under the serum treatment, of which the results are now available for study. One occurred in Greece, the other in the Southwest of the United States. Of the former a set of figures from Athens has been published. The serum employed was chiefly that prepared at the Rockefeller Institute and the cases were treated at the Annunciation Hospital by Dr. Chrestomanós. The tabulation which follows gives the main important facts which are in complete accord with the corresponding data of our previous tabulation (table II).

TABLE VII.

Serum-Treated Cases of the Grecian Epidemic, 1911–12.

Period of injection.	No. of cases.	Recovered.	Died.	Per cent. died.
1st to 3d day	100	87	13	13.0
4th to 7th day	54	40	14	25.9
Later than 7th day	32	17	15	47.0
Totals	186	144	42	22.6

During the past two winters (1911–12, 1912–13) Texas, Louisiana, and still other southern and western states have suffered in some degree from an extension or reappearance of epidemic meningitis. The serum was employed in many of the cases, and the reports from Texas[5] and Louisiana are available for 1911–12. They are specially valuable since they indicate what can be accomplished by the employment of the serum not only inside hospitals but outside and, as often happens, under the disadvantages of poor surroundings and, doubtless, by physicians without previous experience in the performance of lumbar puncture.

The total cases reported from the cities (exclusive of Dallas) and counties in Texas numbered 1,956. Of them 562 received no serum and the mortality was 77 per cent. 1,394 were given one or more injections of the serum and the mortality was 37 per cent. Since the conditions for the care of patients tend to be better in the cities than in country districts, any treatment is likely to be carried out more effectively in them. The figures for three cities that suffered heavily in the epidemic follow.

[5] Sophian, A., Experimental Cerebrospinal Meningitis, St. Louis, 1913.

Galveston: 83 cases treated with the serum; mortality 24 per cent.
Houston: 169 cases treated with the serum: mortality 27 per cent.
Dallas[6]: 444 cases treated with the serum; mortality 27 per cent.
Dallas[7]: 180 cases treated with the serum; mortality 25 per cent.

The preceding figures are uncorrected and include all cases receiving the serum without reference to the quantity given or the period of the disease when first treated. Not a few cases came under treatment when already moribund. Among the group of cases observed by Dr. Sophian 19 were in a dying condition when first given the serum and survived the injection less than twenty-four hours. Excluding these almost hopeless examples lowers the mortality among the 161 remaining cases to 15.5 per cent.

TABLE VIII.

Shreveport Epidemic, 1912.

	Serum-treated.				Non-serum-treated.			
	Total No. of cases.	Recovered.	Died.	Per cent. died.	Total No. of cases.	Recovered.	Died.	Per cent. died.
	176	123	53	30.1	74	11	63	85.0

Mortality according to Race.

	Serum-treated.				Non-serum-treated.			
	Total.	Recovered.	Died.	Per cent. died.	Total.	Recovered.	Died.	Per cent. died.
White.....	67	47	20	29.8	27	7	20	74.0
Colored.....	109	76	33	30.3	47	4	43	91.5

The data given in the official report of the Louisiana State Board of Health relating to the Shreveport epidemic may be studied from various points of view. The total cases numbered 250. The results of my analysis of the cases are presented in the tabulation. The chief facts may be stated as follows: the mortality among the non-serum-treated cases was 85 per cent., and among the serum-treated 30 per cent.; white and colored patients responded equally to the serum, but without serum the colored patients succumbed in

[6] Reported by Dr. Nash.
[7] Reported by Dr. Sophian.

greater proportion than the white. Doubtless this difference arose from the generally poorer surroundings and care of the colored race rather than from any inherent lack of resistance to the infection. As a rule, the number of injections and the amount of serum administered have been given in the report. Where the administration was obviously futile the case has been reclassified.

CONCLUSION.

The data brought together in this report have been gathered from a wide territory and for a period extending over several years. The antimeningitis serum was first employed in 1906 and the latest figures relating to its use included in this report were furnished in 1912. There is no longer doubt that the serum has come to be applied under conditions fairly representing all known manifestations of epidemic meningitis. Hence the test of the serum treatment may be regarded as having been a rigorous one. The initial difficulties surrounding the administration by direct subdural injection have been largely overcome and doubtless will be still further mastered. Already the serum is being successfully applied in private as well as in hospital practice. This gain will probably be reflected in a still further diminution of the mortality since early injection plays such a large part in determining the results achieved.

The 1,300 cases studied in this report are a part only of a far greater number of cases actually treated with the serum supplied by the Rockefeller Institute. It was not found possible to secure histories of all the cases treated; but there is no reason to suppose that the results of the analysis would have been essentially different if reports of a still larger number of cases had been returned. The decision arrived at is not based upon statistical computations alone, but upon objective data as well that are not readily misinterpreted.

The conclusion that follows was first stated in 1909 as the result of an analysis of 400 serum-treated cases. It is supported by the study of the larger series of cases just presented.

"In view of the various considerations presented, the conclusion may be drawn that the antimeningitis serum, when used by the subdural method of injection, in suitable doses and at proper intervals, is capable of reducing the period of illness; of preventing, in large

measure, the chronic lesions and types of the infection; of bringing about complete restoration of health, in all but a very small number of the recovered, thus lessening the serious, deforming, and permanent consequences of meningitis; and of greatly diminishing the fatalities due to the disease."

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PARALYSIS IN A DOG SIMULATING POLIOMYELITIS.*

By SIMON FLEXNER, M.D., and PAUL F. CLARK, Ph.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research, New York.*)

Plates 103 to 107.

The specimen about to be described[1] consisted of the spinal cord and intervertebral ganglia of a paralyzed dog removed from the recently chloroformed animal. Its history in brief is as follows:

The animal, a brindle Boston bull between four and five years old, was noted to have difficulty in swallowing; the mucous membrane of the throat was not red. The next day the left fore leg was lame and the animal was suspected of developing rabies. There was little fever, if any. The brain functions were normal. On the fourth day the left fore leg was completely flaccid; the head drooped and was raised with difficulty. The angles of the mouth drooped also. The paralysis progressed somewhat further in the muscles of the neck, and the right leg showed indefinite involvement. The dog was chloroformed on December 27 and the vertebral column was removed and brought to the Rockefeller Institute where the spinal cord and ganglia were taken out aseptically.

Dr. Zucker expressed the opinion that the paralysis in the dog resembled the paralysis of poliomyelitis in man and differed from the common distemper paralysis. This opinion is supported by the autopsy findings and microscopical examination.

The lesions in the spinal cord were obvious on gross examination and consisted of swollen, softened, and hemorrhagic foci in the cervical, and to a less extent in the lumbar region. Portions of the spinal cord were placed in sterile 50 per cent. glycerin, and on December 7 two puppies and two *Macacus rhesus* monkeys were inoculated with an emulsion prepared from the affected parts of the cord. One each of the dogs and monkeys was inoculated respectively intracerebrally and into the sciatic nerve and peritoneum. The inoculations were made under ether anesthesia and produced no result.

* Received for publication, March 1, 1913.

[1] We are indebted to Dr. F. A. Zucker, of Elizabeth, New Jersey, for this interesting specimen.

The inoculated animals remained under observation for several months and developed no symptoms of paralysis.

The failure of the inoculations centers the direct interest upon the pathological changes in the spinal cord and intervertebral ganglia. They are pronounced. No part of the cord is free from changes and the most pronounced lesions are present in the cervical and lumbar enlargements. The lesions are not diffuse but they affect chiefly the anterior, and to a less extent the posterior gray matter. The white matter has escaped except to the extent that the contained blood vessels are often affected. The lesions consist of (*a*) perivascular infiltrations, (*b*) hemorrhage, (*c*) edema, infiltration, and necrosis of the ground substance followed by an invasion of large phagocytic cells (compound granular corpuscles), and (*d*) necrosis of ganglion cells which become, at times, replaced by small round cells. The lesions in the intervertebral ganglia consist of perivascular infiltration and accumulation of round cells about the ganglion cells, which rarely are degenerated. The perivascular infiltration is present in the nerve roots, but is less marked there than in the ganglia or spinal cord. The most pronounced lesions are about the blood vessels within the spinal cord, although vessels in the meninges not infrequently show marked infiltrations (figure 1). The largest arteries and veins tend not to be affected and the infiltration is most apparent about the intramedullary vessels at the margin of and within the gray matter (figure 2). The largest accumulations of cells are anterior, possibly because the vessels are of larger caliber, but arterioles and venules show some degree of cellular invasion. From the vessels the ground substance is invaded irregularly, and in many places the invading cells have surrounded ganglion cells showing more or less degeneration (figure 3).

The hemorrhages are small and confined to the immediate locality of blood vessels. But the edema extends at times throughout the gray matter. As a rule, the anterior horns are affected disproportionately. The rarefied ground substance stains lightly and is poor in cells except about the vessels and infiltrated ganglion cells. Focal necrosis of the gray matter involving ground substance, vessels, and nerve cells was confined to the cervical and lumbar enlargements. In the former it corresponded with the paralysis of the fore leg and

neck observed during life, and in the latter it was less extensive although still taking in a large fraction of the affected anterior horn. Very little of the original tissues remained except the coarser framework which now supported large numbers of phagocytic cells (figure 4).

Degeneration of the nerve cells occurred at all levels, but was most pronounced in the cervical and then in the lumbar region. The degenerated cells lacked protoplasmic processes and tigroid substance, were homogeneous and acidophilic and often shrunken. All stages of the degenerative process could be followed in different sections. About these nerve cells lymphoid cells accumulated, but rarely, if ever, invaded, although frequently taking the place of, the degenerated nerve cells. Neurophagocytosis was absent.

The infiltrating cells about the blood vessels and within the gray substance, excluding the phagocytic cells, were of the mononuclear character. They have the size and general appearance of cells of the lymphoid type (figure 5). Polynuclear cells appear to have been wholly absent even from the larger necrotic foci.

The lesions of the intervertebral ganglia are chiefly perivascular. The cellular infiltration is identical with that of the spinal cord except that the degree is less (figure 6). From the vessels the neighboring framework is invaded and the ganglion cells are surrounded. Rarely the pericellular infiltration is great enough to cause degeneration of such a cell (figure 7). Within the nerve roots the blood vessels show a similar, if slighter, grade of cellular infiltration (figure 8).

There is undoubted similarity in the pathological condition described with the lesions occurring in the spinal cord and intervertebral ganglia in poliomyelitis. The lesions in the cord and ganglia of the dog are, however, not identical with those of poliomyelitis in man or the monkey. The perivascular infiltration and the edema, hemorrhage, and necrosis of the gray substance of the cord are in close agreement, but the degeneration of the nerve cells is different. In poliomyelitis the necrotic nerve cells disappear less by a process of autolysis, but are removed more actively by phagocytes which may account for the distinct difference in appearances presented by the degenerating cells in the two conditions.

However, it is obviously improper to generalize from a single example, especially since the resemblances between the lesions in the dog and those characteristic of poliomyelitis are more numerous than the divergences. It would also be unsafe to conclude from the negative inoculations that the paralytic condition in the dog is of non-communicable character. The lesion in the spinal cord may well not have been as recent as the clinical history suggests, so that the most favorable period for the transmission of the disease may have passed at the time the inoculations were made. This important point, as well as the fact of the precise nature of the malady causing the lesions and the paralysis, still call for elucidation.

There is a well defined nervous disease of large domestic animals with which the condition of the dog bears some resemblance. In Borna's disease of horses, an extensive perivascular, mononuclear cellular infiltration occurs leading to paralysis and death. The lesions occur invariably in the brain and inconstantly in the spinal cord. From the vessels the ground substance of the nervous tissues may become infiltrated with cells, but the nerve cells show inconspicuous changes and, apparently, never severe degeneration or necrosis. The central nervous organs of man in sleeping sickness show a similar high degree of perivascular mononuclear infiltration most marked in the brain. Whether in this dog the brain was affected cannot be known as it was not submitted for examination.

CONCLUSION.

A pet dog developed a form of paralysis simulating the paralysis of epidemic poliomyelitis. The autopsy showed changes in the spinal cord and intervertebral ganglia, resembling, but not identical with, the lesions of poliomyelitis in man. Inoculation of the affected spinal cord into the nervous system of young dogs and *Macacus rhesus* monkeys was not followed by the production of paralysis or other obvious symptom of disease.

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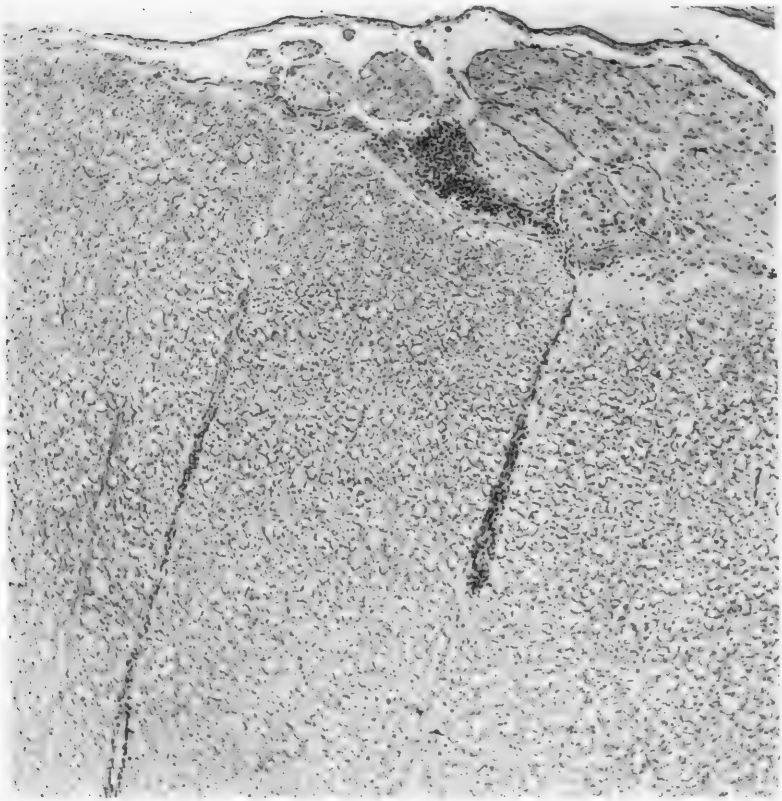

FIG. 1.

(Flexner and Clark: Paralysis in a Dog Simulating Poliomyelitis.)

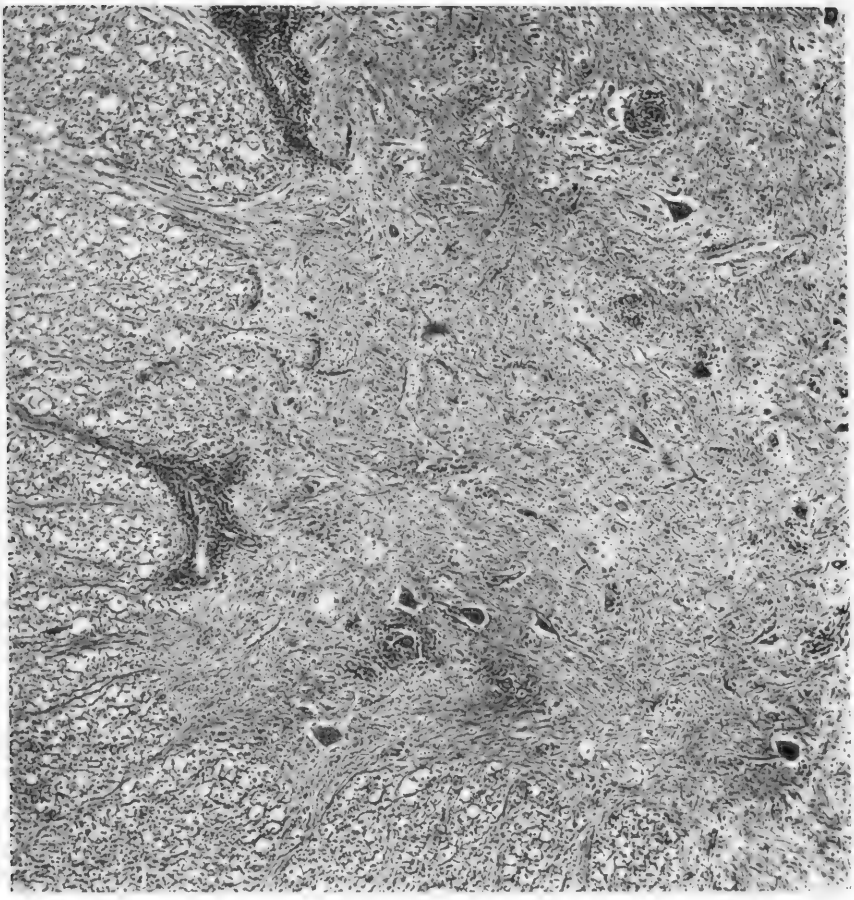

FIG. 2.

(Flexner and Clark: Paralysis in a Dog Simulating Poliomyelitis.)

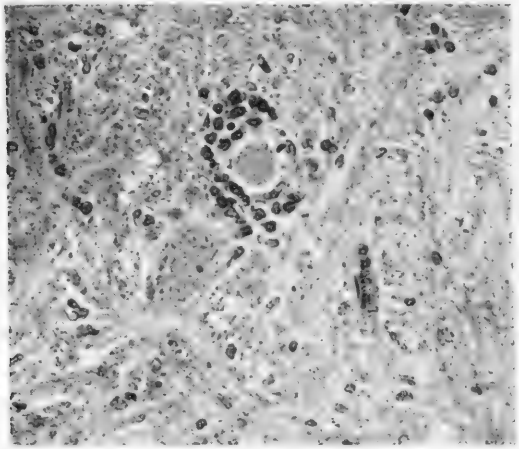

FIG. 3.

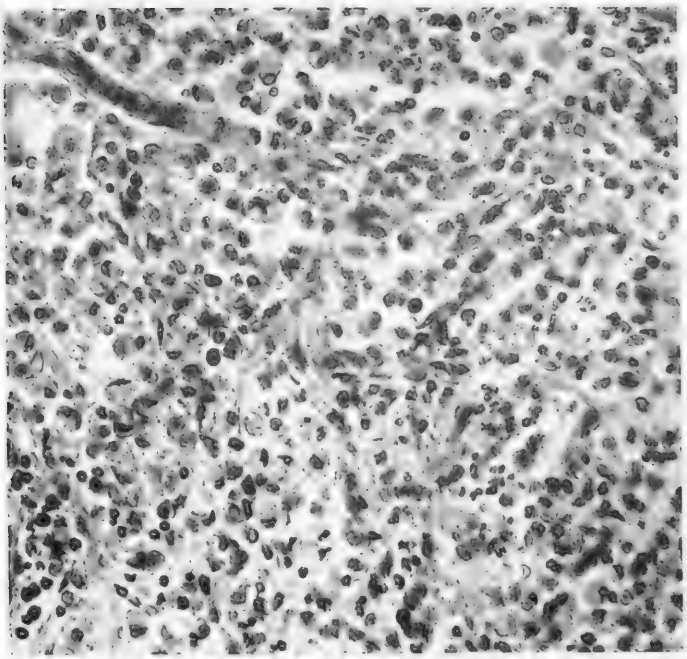

FIG. 4.

(Flexner and Clark: Paralysis in a Dog Simulating Poliomyelitis.)

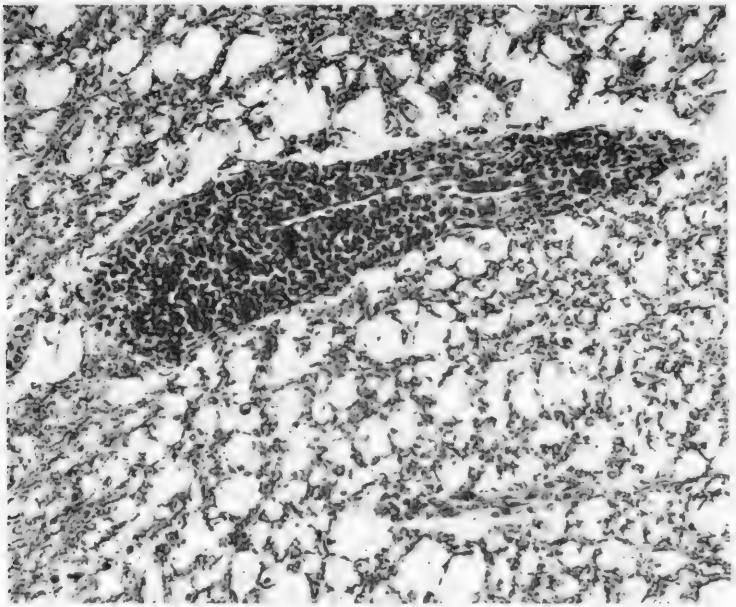

FIG. 5.

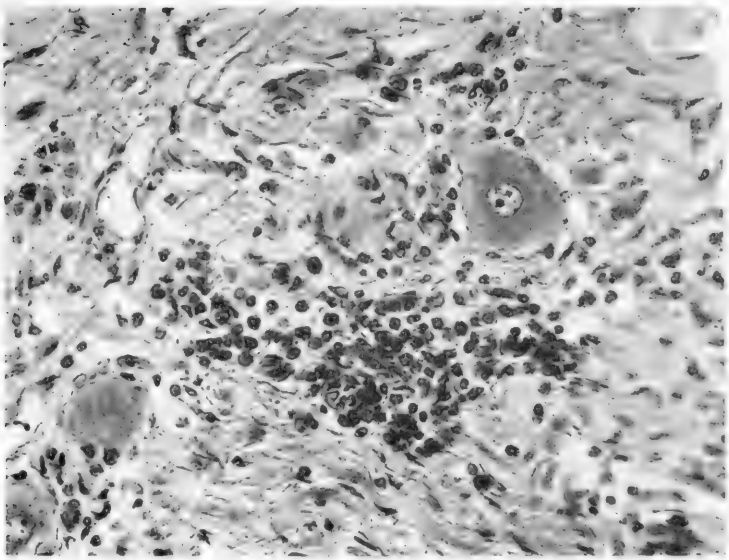

FIG. 6.

(Flexner and Clark: Paralysis in a Dog Simulating Poliomyelitis.)

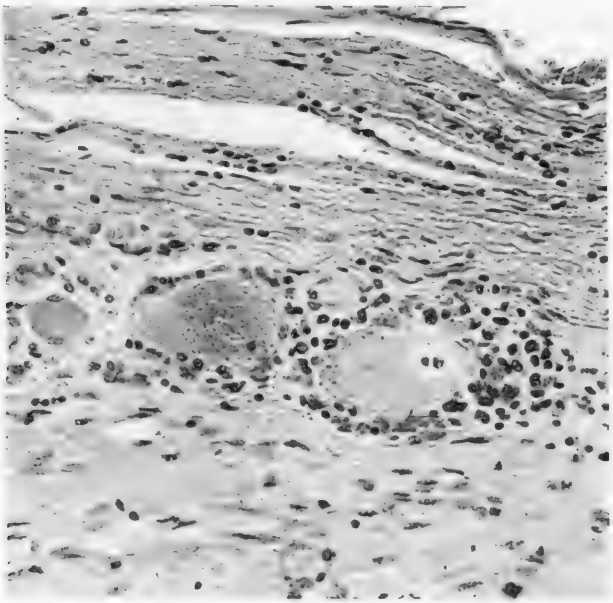

FIG. 7.

FIG. 8.

(Flexner and Clark: Paralysis in a Dog Simulating Poliomyelitis.)

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EXPLANATION OF PLATES.

Plate 103.

Fig. 1. Spinal cord. Section showing lymphoid cell infiltration about vessels in the extra- and intramedullary meninges. × 98.

Plate 104.

Fig. 2. Spinal cord. Section showing extensive lymphoid cell infiltration of the blood vessels at the margin of the anterior horn and also mononuclear cellular collection about nerve cells within the gray matter. × 81.

Plate 105.

Fig. 3. Spinal cord. Lymphoid cell infiltration about the ganglion cell in the anterior horn; the nerve cell shows degeneration. × 330.

Fig. 4. Spinal cord. Phagocytic cells within the degenerated gray matter of the anterior horn. × 330.

Plate 106.

Fig. 5. Spinal cord. Lymphoid cell infiltration about a large vessel in the white matter. × 144.

Fig. 6. Intervertebral ganglion. Lymphoid cell infiltration about a small vessel. × 330.

Plate 107.

Fig. 7. Intervertebral ganglion. Lymphoid cell infiltration about degenerating ganglion cells. × 330.

Fig. 8. Nerve root. Lymphoid cell infiltration about small vessels. × 98.

THE IMMUNIZATION OF LARGE ANIMALS TO A PATHOGENIC TRYPANOSOME (TRYPANOSOMA HIPPICUM (DARLING)) BY MEANS OF AN AVIRULENT STRAIN.*

By S. T. DARLING, M.D.

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The literature on the subject of the curative agents for trypanosomiasis indicates that the arsenic derivatives available at present, while possessing some value in treating small laboratory animals experimentally inoculated, are of little or no value in treating the large animals naturally subject to the disease. Holmes[1] and others have cured cases of surra by means of arsenic or some of its derivatives. But with Holmes's method I have failed completely in murrina, the trypanosomal disease of horses of Panama, for, whenever it was possible by careful dosage to destroy the trypanosomes, the horses or mules always died soon afterwards of arsenic poisoning. Practically all the successful chemotherapeutical applications of arsenic and its derivatives in the treatment of trypanosomiasis have been made with small laboratory animals.

Trypanosomiasis apparently occupies a position between malaria and syphilis with regard to the amount of actively acquired immunity derived from infection. In malaria repeated and continued attacks result in a definite, though not high grade, immunity. Why may not immunity be derived in trypanosomiasis as well, by the production of a low grade infection with an avirulent strain?

The experiments to be described were carried out with a view of showing that immunization of large animals can be effected by means of an avirulent strain of a pathogenic trypanosome

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[1] Holmes, J. D. E., *Parasitology*, 1910, iii, 288.

(*Trypanosoma hippicum*), that immunized animals completely recover from the infection, and that they can not be reinfected by the same strain nor by a virulent strain of the same species. And it is believed that a practicable mode of immunization of large animals to fatal forms of trypanosomiasis can be developed.

The chief objection that may be raised to this method of immunization is the danger from a recrudescence of virulence in the immunizing strain and its subsequent transmission in virulent form to other animals. In the process of immunization, after the acute stage of the disease is over, the trypanosomes are present in such small numbers that it is unlikely that they could be transferred by flies to other animals. As a matter of fact, no secondary cases arose in the corrals during the immunization of the mules in question.

The pathogenic powers of this trypanosome have been established.[2] When the disease was epizoötic in the corrals, all the mules ascertained, by microscopic examination, to be infected either died of the disease, or dying, were shot. Experimentally infected mules and horses, with the exceptions mentioned below, and concerned in the development of immunity by means of the avirulent strain, always died. No other animal was known to recover save one treated with arsenic, and this animal died later of arsenic poisoning.

I have called attention to the possibility of reducing the virulence of a strain of this trypanosome by selection from a guinea pig.[3]

A strain of *Trypanosoma hippicum* that had survived in a guinea pig for the exceptionally long period of 336 days, showed upon subinoculation on the 279th and 336th day feeble pathogenic powers when compared with several other strains and with the same strain at an earlier period of the infection in the guinea pig.

A mule inoculated from this guinea pig on the 279th day became infected after a prolonged incubation period, and recovered from the infection. Three hundred and thirty-six days after inoculation, or on the day of death, a dog was inoculated. It became infected but recovered from the infection.

[2] Laveran, A., *Bull. Soc. path. exot.*, 1911, iv, 168.
[3] Darling, S. T., *Bull. Soc. path. exot.*, 1912, v, 184.

The usual duration of the disease in guinea pigs may be placed between one and four months. The duration of 336 days is, therefore, exceptionally long and indicates either a resistant host or an avirulent strain. The subsequent history of this strain shows positively that it was a weakened one, for it failed to kill two mules, a dog, and a guinea pig. The lives of other animals infected with this strain were uncommonly prolonged. The strain also required a prolonged incubative period when inoculated into mules.

Mule 318.—A mule was experimentally inoculated on November 30, 1910, with this strain from the guinea pig. At the same time two other mules were inoculated as controls with virulent strains as follows:

	Strain of reduced virulence.	Virulent strains.	
Hoof No.	318	113	269
Date of inoculation.....	Nov. 30, 1910	Nov. 30, 1910	Nov. 30, 1910.
Incubative period.....	11 days	8 days	8 days.
Duration of disease.....	Recovered	44 days	105 days.
Remarks	Treated with arsenic	Treated with arsenic	Treated with arsenic.

It is seen that the period of incubation was prolonged to eleven days in the case of the animal infected with the strain of reduced virulence, the usual period for mules being from six to eight days. Although each animal was treated with arsenic according to Holmes's method, the only animal that recovered was the one inoculated with the strain of reduced virulence.

In spite of the arsenic medication trypanosomes remained in the mule's blood for six months after arsenic had been discontinued, although they were only demonstrable by animal subinoculation, indicating that arsenic had no influence in effecting the recovery of the mule. The mule was definitely infected and passed through a typical attack of the disease, becoming very much emaciated, during which time trypanosomes could be demonstrated in the peripheral blood by microscopical examination. In April, 1911, it grew fat and appeared in good condition, though hemagglutination was noted in blood films as late as August 16, an evidence of subliminal or latent infection. All the small animals subinoculated on August 16 became infected. The mule's condition continued excellent,

and on January 8, 1912, over one year after his infection by the avirulent strain, hemagglutinins had disappeared from blood films and ten cubic centimeters of blood inoculated into a white rat and a guinea pig did not result in infection. The mule was apparently cured.

Mule 92.—This mule was infected by means of *Musca domestica* with the same avirulent strain from a guinea pig that had been inoculated from mule 318 on August 16. The date of inoculation of the mule was November 28, 1911. The period of incubation was prolonged to ten days, showing that the strain was avirulent. The animal passed through a typical attack, trypanosomes disappeared from the peripheral blood but were demonstrable by animal subinoculation on March 16, and his temperature became normal on April 1, when all symptoms disappeared. Neither hemagglutination nor trypanosomes could be detected on July 16. The animal's temperature was normal and his condition was excellent. Three animals, a guinea pig, a white rat, and a white mouse, were subinoculated from the mule on July 16, and all became infected. On this date, after removing blood for subinoculations, the mule was reinoculated with the same avirulent strain to ascertain whether it would cause febrile or other manifestation, but none was noted. On August 7 the mule was reinoculated with a virulent strain of *Trypanosoma hippicum* more recently obtained from a fatal case of the disease in a Panaman pony, but there were no symptoms, rise in temperature, hemagglutination, or trypanosomes. On August 16 a longitudinal strip of edema on the lower belly wall appeared, but neither trypanosomes nor hemagglutination could be detected. On October 30 the animal was fat and sleek and in excellent physical condition, and blood was negative for trypanosomes upon subinoculation of 8 c.c. into a guinea pig. On December 13 the animal's condition continued excellent, and the blood was negative for trypanosomes upon subinoculation of 5 c.c. into a white rat. On January 15 the mule had been at work for four weeks.[4]

In each experiment there is a return to normal physical condition and recovery from infection, and the animals were demonstrated not to be infected by animal subinoculations. The recovery cannot be attributed to the effect of arsenic medication on the strain while in mule 318, for the strain while in the original guinea pig was avirulent for the dog and guinea pig before being subjected to arsenic medication in the mule.

After hemagglutination disappeared from blood films, the phenomenon did not reappear, and after trypanosomes disappeared from blood films, they did not reappear, although upon subinocu-

[4] On April 7 the mule is still in excellent condition and is hard at work in a quarry.

lation into susceptible animals the blood was demonstrated to be infected.

One animal, specially tested, was observed to be immune to the same strain and resisted infection to a virulent strain as well. It is believed that the production of subliminal infections by means of avirulent strains will yield efficient immunity against subsequent infection from a virulent strain, and the danger from a recrudescence of virulence within the immunized animal and the infection of other animals would appear to be nil.

THE ACTION OF PNEUMOCOCCUS ON BLOOD.*

By E. E. BUTTERFIELD, M.D., and F. W. PEABODY, M.D.

(From the Laboratories and Hospital of The Rockefeller Institute for Medical Research, New York.)

During the winter of 1911 one of us (Peabody) made the observation that in some cases of lobar pneumonia the oxygen capacity of the blood is reduced below the normal level without a corresponding diminution of the hemoglobin content (Sahli hemoglobinometer). Several possibilities suggested themselves: (1) the presence of reducing substances in the serum; (2) a shift of the equilibrium between oxygen and hemoglobin, due possibly to an increase in the concentration of $H^{\cdot}$ ions in the serum; (3) the formation of some derivative of hemoglobin which does not combine with oxygen. Experiments with washed corpuscles from these patients showed that a reduction of the oxygen capacity still persisted after removal of the serum. This result eliminated from consideration the presence of reducing bodies and a shift of equilibrium due to some constituent of the serum, and led us to regard the diminution of the oxygen capacity as the manifestation of some irreversible constitutive change in the oxyhemoglobin within the red cells. It was soon found that a similar drop in the oxygen capacity of the blood occurred in guinea pigs and rabbits with pneumococcus infection, and, furthermore, that the action of pneumococcus cultures on washed rabbit corpuscles *in vitro* also produced constantly a definite drop in the oxygen capacity and light absorption. This gave us a convenient means of studying the phenomenon under varied and controllable conditions, with the object in view to determine, if possible, what derivative of hemoglobin was formed in the process.

The method decided upon was to take equal volumes of washed rabbit corpuscles and to add to one portion, which was to serve as

* Received for publication, March 10, 1913.

a control, a known volume of 0.9 per cent. sodium chloride,[1] and then to add the same volume of a bouillon culture of virulent pneumococcus to the other portion of washed rabbit corpuscles. Both preparations were kept at 37° C., usually for eighteen hours, after which they were laked with equal volumes of distilled water and centrifuged. In the clear supernatant fluid of control and pneumococcus preparation the oxygen capacity (Haldane-Barcroft method) and the light absorption (spectrophotometer of Martens, König, and Grünbaum) were then directly determined.[2] Both preparations were vigorously shaken with air before any measurements were made.

There exists between the light absorption in two regions of the spectrum and varying concentrations of oxyhemoglobin in the presence of some other derivative of hemoglobin a mathematical relation. We shall not go into the derivation of the formula here, but merely state the relations in their final form. For the relation between the light absorption of a mixture and the relative concentration of oxyhemoglobin (or of the second derivative), the sum of both concentrations being taken equal to 1, one finds:

$$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}} = \frac{(\beta_1 - \alpha_1)x + \alpha_1}{(\beta_2 - \alpha_2)x + \alpha_2}. \qquad (1)$$

I_1 and I_1' are, respectively, the intensities of the light entering and leaving the solution at a given wave length in the spectrum, α_1 is the absorption constant of oxyhemoglobin, and β_1 the absorption constant of the second derivative at the same wave length. I_2, I_2', α_2, and β_2 are the corresponding quantities at another wave length in the spectrum, x is the relative concentration of the second derivative, and $(1 - x)$ would be the relative concentration of oxyhemoglobin.

For the relation between the absolute concentration of oxyhemoglobin in the presence of another substance with optical constants

[1] Sterile bouillon was also used as a diluent for the control in some experiments, without appreciably affecting the results.

[2] For measurement of the light absorption in concentrated solutions of oxyhemoglobin see Butterfield, E. E., *Ztschr. f. physiol. Chem.*, 1912, lxxix, 439.

β_1 and β_2 and the light absorption at two different wave lengths, one finds:

$$c_1 = \frac{2.30\left(\beta_2 \log \frac{I_1}{I_1'} - \beta_1 \log \frac{I_2}{I_2'}\right)}{l(\alpha_1\beta_2 - \alpha_2\beta_1)}. \qquad (2)$$

Here c_1 is the absolute concentration of oxyhemoglobin and l linear thickness of the absorbing layer. The other symbols have the same significance as in formula (1).

To apply these formulas to the present problem it would be necessary to know the optical constants of oxyhemoglobin and those of the second derivative of hemoglobin. But the second derivative is one of our unknowns. It would, therefore, be necessary to take the measured values for the light absorption and try out a series of analogous formulas in all of which the optical constants of oxyhemoglobin figure, but in each of which a different set of constants corresponding to the different derivatives of hemoglobin occurs. If a formula is found which gives, for the change in the oxyhemoglobin concentration of the pneumococcus preparation as compared with the control, values agreeing with those for the change in the oxygen capacity, the second derivative is then identified with a high degree of probability. Our course was fortunately much shortened by the observation that when pneumococcus culture is incubated with laked blood there appears spectroscopically an absorption band in red. When washed corpuscles are used the process rarely progresses to such an extent that an absorption band in red becomes visible. This narrowed the choice of the second derivative down to methemoglobin or hematin. Furthermore, Grüter[3] has made excellent spectroscopic observations on blood dropped into pneumococcus cultures. From the appearance of the absorption band in red and from the spectroscopic changes on reduction he reached the conclusion that some substance in a pneumococcus culture converts oxyhemoglobin into methemoglobin.[4] Therefore

[3] Grüter, W., *Centralbl. f. Bakteriol., 1te Abt., Orig.*, 1900, 1, 241.

[4] This method is not entirely free from objection, because the spectrum of the reduction product of hematin, hemochromogen, in relatively small quantities, would be masked by the diffuse absorption of reduced hemoglobin. It would be thus impossible to distinguish hematin from methemoglobin in the presence of oxyhemoglobin except in the case of relatively large amounts of hematin.

we were led at first to use the optical constants of methemoglobin together with those of oxyhemoglobin in the formula for the light absorption as a function of the concentration. Accordingly, crystalline oxyhemoglobin and methemoglobin were prepared from ox blood and the light absorption was determined in dialyzed solutions at the wave lengths 577, 579 $\mu\mu$ (double line, mercury lamp), 546 $\mu\mu$ and 436 $\mu\mu$. For the present work the constants at 577, 579 $\mu\mu$ and 546 $\mu\mu$ were used. The values are for oxyhemoglobin $\alpha_1 = 21.00$ and $\alpha_2 = 18.42$, for methemoglobin $\beta_1 = 5.18$ and $\beta_2 = 7.31$.

The first table represents data collected to show the degree of agreement between the gasometric and photometric measurements for widely varying values of the relative concentration, x. In table I the percentage concentration $100x$ is used.

TABLE I.

	Control.		Pneumococcus preparation.		Per cent. diminution of oxygen capacity.	Per cent. diminution of oxyhemoglobin concentration.	Nature of experiment.
	Oxygen capacity.	$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$	Oxygen capacity.	$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$			
I	3.26	1.14	2.93	1.12	10	11	Washed rabbit corpuscles + bouillon culture pneumococcus + sodium hydroxide.
II	3.40	1.14	2.87	1.11	16	16	Washed rabbit corpuscles + bouillon culture pneumococcus.
III	16.96	1.15	12.98	1.10	24	21	Washed rabbit corpuscles + bouillon culture pneumococcus.
IV	5.92	1.14	4.36	1.08	26	29	Washed rabbit corpuscles + bouillon culture pneumococcus.
V	3.65	1.14	1.09	0.96	70	64	Laked rabbit blood + bouillon culture pneumococcus.
VI	3.40	1.14	0.37	0.79	89	91	Laked rabbit blood + bouillon culture pneumococcus.

The agreement is excellent in most cases, but can only be realized in the case that the dilutions are so accurate that control and pneumococcus preparation have the same initial concentration of oxyhemoglobin. The difficulty in obtaining this lies in the sedi-

mentation of the red cells. Formula (2), which gives the relation between the absolute concentration of oxyhemoglobin and the change in the light absorption, is independent of dilution. We give two examples of the use of this formula.

TABLE II.

Relation between Oxygen Capacity and Absolute Concentration of Oxyhemoglobin.

		$\log \frac{I_1}{I_1'}$	$\log \frac{I_2}{I_2'}$	c_1 in gm. per 100 c.c.	Oxygen capacity, volume per cent.	Per cent. diminution in	
						c_1	Oxygen capacity.
I	Control	0.523	0.457	2.72	3.84	14	15
	Culture	0.466	0.418	2.34	3.27		
II	Control	0.437	0.385	2.30	3.26	32	32
	Autolysate	0.321	0.292	1.57	2.23		
	Thickness of light-absorbing layer = 0.021 cm.						

Here it is seen that the agreement between gasometric and optical results is even better than in the first series. It will be noticed from the second experiment of the second series that the autolysate of pneumococcus cultures, which in this case was filtered and sterile, also produces the same effect on blood as does the growing pneumococcus.

The identification of the substance or substances in the pneumococcus cultures, which induces the formation of methemoglobin, is a problem in itself and one into which we do not care to enter here. However, in view of the well known acid production by the pneumococcus it seemed worth while to try the effect of acid on the oxygen capacity and light absorption of the blood, even though our pneumococcus-blood mixtures never showed a definite acid reaction to litmus. Table III gives the result of some experiments in this direction.

It is evident from the results that amounts of acid, which in proportion to the blood volume would be considerable for the living organism, have but little effect on the oxygen-combining power of the blood, and this not at all in the direction of a diminution of the oxygen capacity.

TABLE III.

Acid Series.

	Oxyhemoglobin concentration.	Oxygen capacity.	$\frac{\text{Oxygen capacity}}{\text{Oxyhemoglobin concentration.}}$	Quantity of n/10 hydrochloric acid added to 4.5 c c. of washed rabbit corpuscles, in c.c.
I	4.27	5.97	1.40	0.0
II	4.47	6.25	1.40	0.5
III	4.48	6.37	1.42	1.0
IV	4.40	6.35	1.44	2.0

CONCLUSION.

The reduction of the oxygen capacity which occurs after incubating pneumococcus cultures with washed rabbit corpuscles is due to the formation of methemoglobin (or some derivative of hemoglobin with identical optical constants for three regions in the spectrum). The substance which induces the change is also present in the sterile filtrate of autolyzed cultures. By analogy we feel justified in concluding that the mechanism of the reduction of the oxygen capacity in human lobar pneumonia is at least in part of the same nature. To determine the frequency and intensity of the phenomenon in lobar pneumonia, and thereby to establish its clinical significance, is the next step and a problem upon which we are now engaged.